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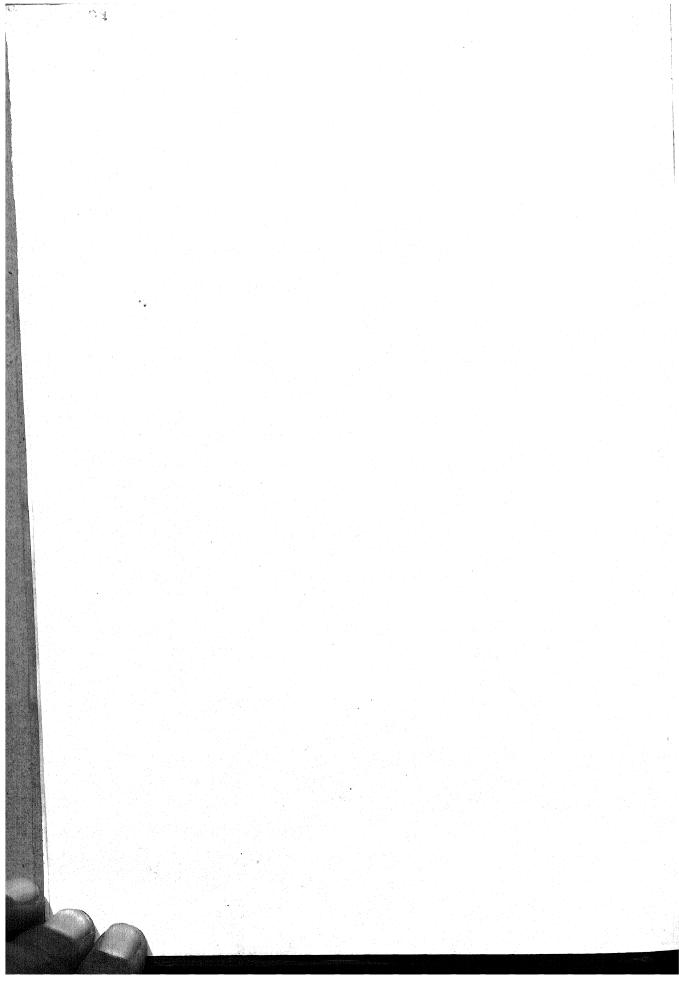
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# ERRATA

Page 413, table 2, heading of last column.—Substitute symbol "o" for "? o"." Page 536, column 2, line 44.—Substitute "Kaweah" for "Kern."



# STUDIES IN THE GENETICS AND CYTOLOGY OF TWO SPECIES IN THE GENUS ASTER AND THEIR POLYMORPHY IN NATURE <sup>1</sup>

Ralph H. Wetmore and Albert L. Delisle

THE FOLLOWING genetical and cytological studies were made in conjunction with investigations in relative growth and morphogenesis (Delisle, 1938) in two species of Aster, A. novae-angliae L., A. multiflorus Ait.2 and their hybrid. Certain aspects of this work appear significant when considered in relation to the polymorphy of these species and possibly have a bearing on the recognized polymorphy in the genus. A. novae-angliae is tall, hairy, stout, and relatively unbranched in the young stages, becoming corymbed only at the summit later. It has numerous large, soft, minutely pubescent, lanceolate, acute, entire leaves which are provided with marked auriculations at the base. The bracts of the involucre are loose, erect, nearly equal, glandular-viscid and linear; the heads are characteristically solitary on individual branchlets; the rays of the type species are violet-purple and those of the forms roseus (Desf.) Britton and geneseensis House are rose and white, respectively. This species flowers from August to October, with the bulk of the plants blooming in September.

A. multiflorus is a somewhat shorter, very much more branched (Delisle, 1937) and bushy, short-pubescent plant with crowded heads on the spreading, racemose branches. The leaves are crowded, linear, entire, sessile, non-auriculate, spreading, and rigid, with rough or ciliate margins, passing above into the spatulate, obtuse, ciliate bracts of

<sup>1</sup> Received for publication September 6, 1938.

This is one of a series of studies on the genera Aster and Solidago which were begun when the senior author was a National Research Fellow in the Biological Sciences. To the Council he expresses his gratitude for their support at that time.

<sup>2</sup>The name A. multiflorus is used here because of its general occurrence in Gray's Manual, 7th edition, and other floras, instead of A. cricoides, which, according to Mackenzie, antedates it and under which the original description was made. (Mackenzie, 1926; Blake, 1930.)

the inflorescence; the involucral bracts are hairy and have squarrose or spreading tips; the rays are white on numerous, small, crowded, racemose heads. Flowering occurs from August to October. A comparison of some of the characters of this species is made with those of A. novae-angliae in table 1.

The hybrid between the two species is intermediate in many characters, upright, much branched, but not bushy, puberulent to hirsute, but not glandular, with soft leaves and involucral bracts merely spreading at the tip. It flowers slightly later,

mostly in September to October.

Figure 1a indicates the relative size of the heads in A. novae-angliae and A. multiflorus. In this figure are shown also the contrastive sizes and numbers of rays in the capitulum of each species. The contrast of achene shape and size is evident from figure 1b. Figure 2 illustrates the nature of leaves and branches in the two species of Aster and their hybrid.

Concurrently with the genetical study of these two species of Aster, a cytological investigation of the same material was undertaken. It included studies in the number, morphology, and behavior of the chromosomes during mitotic and meiotic divisions (the pollen mother cells) in these species, their varieties, their reciprocal hybrids, the back crosses, and plants of the  $F_2$  generation. Investigations were carried out on field material whenever available. The study also included an ecological variant, a forma depressa of A. multiflorus.

MATERIALS AND METHODS.—Test experiments carried out on many plants of these two species over a period of years proved them to be self-sterile. Repeated controlled pollinations from the same plant have consistently failed to produce achenes.<sup>3</sup> The

<sup>3</sup> The flower-heads of all the plants used were carefully bagged previous and subsequent to anthesis in order to eliminate all sources of contamination. The pollen in all

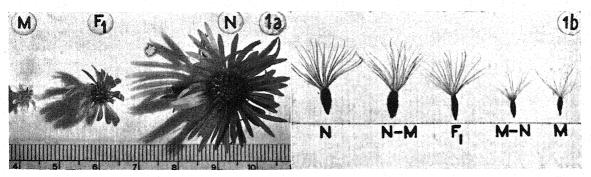


Fig. 1a (left). Relative sizes of heads of Aster multiflorus (left), Aster novae-angliae (right), and their hybrid (center).—Fig. 1b (right). Relative size and shape of achenes in Aster crosses. M represents an achene of A. multiflorus; N, A. novae-angliae;  $N \times M$  and  $M \times N$ , achenes which will produce the reciprocal  $F_1$ -hybrids;  $F_1$ , achene produced by the  $F_1$ -hybrid when inbred ( $\times 4.2$ ).

[The Journal for December (25: 727-816) was issued January 11, 1939.]

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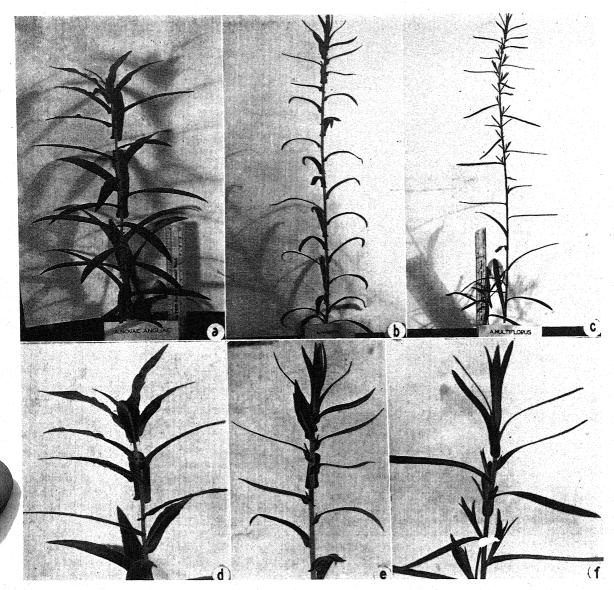


Fig. 2. Morphological comparison of A. novae-angliae (a and d), A. multiflorus (c and f), and their hybrid A. novae-angliae  $Q \times A$ . multiflorus  $\mathcal{E}$ , (b and e). Note differences in branching, auriculation, size of leaf, etc.

breeding experiments reported here are based on the typical A. novae-angliae, with violet-purple rays, and its two forms roseus and geneseensis with rose and white rays, respectively, and the manyflowered white aster, A. multiflorus. The original cases was obtained from bagged flower-heads, transferred on the sterilized blade of a fine iridectomy scalpel, and gently rubbed on the inner surface of the bipartite stigma. Since the marginal ligulate flowers are pistillate, and since anthesis proceeds centripetally in the flower heads of the species, the female ray florets proved more accessible for manipulation and more desirable for contro ed pollina-tion than the centrally located and perfect disc florets. Fertilization must have been effected more frequently in the ray florets, as those produced the largest number of achenes. All achenes were germinated in Petri dishes lined with moist filter paper at room temperature, and the seedlings were transferred to individual small pots in the greenhouse to ensure absolute identity.

material was collected in the field and inbre or two or more generations. Taxonomically these two species of Aster are regarded as being closely related. Genetical are refertile and cross readily with the cross A. novae-ang that the cross A. novae-ang that the cross A. novae-ang that the cross are different several and red achieves were obtained which produced health a dingr. Seventy-fix per cent of these were flower in the sarry year.

The reci tempted, u.

A. mu. florus ways a collected in Combides on wester

4 These were so collected in Cambridge on waste ground behing. It man Ir mary. The forms geneseensie was cotained from achenes produced on he pollinated plants of the white-flowered form a owing the Marvard Bot nic Gardens.

erably less success. Repeated pollinations, however, have produced approximately one hundred achenes, which germinated badly and produced seedlings which are greatly retarded, look somewhat abnormal, and remain in the rosette condition for a longer time (fig. 3b).

Since the interfertility of the  $F_1$  plants is fairly high, there is no difficulty in obtaining achenes for plants of the  $F_2$  generation. Both back-crosses

were also obtained without difficulty.

In the cytological studies, capitula of the proper stage of development were fixed in acetic-alcohol—30 per cent glacial acetic acid and 70 per cent absolute alcohol—and transferred to 85 per cent alcohol, there to remain until examined. Smearing techniques were found not too satisfactory because of the small size of the chromosomes. Resort was therefore made to embedding and sectioning in celloidin (Jeffrey, 1928) according to the technique and schedule outlined by one of the authors (Wetmore, 1932).

Observations.— $F_1$  plants produced from these crosses are generally intermediate in character between the two parents. The stem leaves are distinctly clasping at their bases and are provided with auriculations, as are those of A. novae-angliae. The plants vary in height from 0.45-1.7 m. (table 1).

The hybrid has the characteristic type of glandular pubescence of A. novae-angliae, though the glands are less frequent (see table 1). Breeding experiments have shown the ray color in the type species A. novae-angliae and its two forms to be genetically distinct. When purple-rayed plants are pollinated with pollen from other purple-rayed plants, only purple-rayed individuals are produced; the white-rayed form, when pollinated from whiterayed types, yields only plants with white rays. Further controlled crosses involving the purplerayed and the white-rayed forms have yielded only purple-rayed heads illistinguishable from the type plant and suggesting a complete dominance of color over the albino. The rose-colored form is not so simply interpreted. Further work is necessary before the results obtained can be understood.

The hybrids produced from a cross between A. nover-angliae  $\mathcal{P}$  type with purple rays  $\mathcal{L}$   $\mathcal{L}$   $\mathcal{L}$   $\mathcal{L}$  have clear bluish-violet rays. Crosses involving A. novae-angliae forma roseus  $\mathcal{L}$   $\mathcal{L}$ 

As previously pointed out, the syprids resulting from the cross A multinorunt  $\times$  A. novae-angliae of are greatly retarded. It will be noted that the rosette leave derities in development and in general angles of being

figure are exactly the same ago yorids in this figure are exactly the same ago yorids in this under the same greenhour condain. These hydrogeneous seedlings are not only retarded in their declepment, but are markedly smaller than the

reciprocals of the same age. They possess small cotyledons like those of their female parent, as shown in table 1. Comparable results were obtained in mice by Castle (1936) and in Triton by Pariser (1936).

Table 1 and figure 1b give the relative sizes of the achenes from which these reciprocal hybrids germinated. It will be noted that the differences



Fig. 3. Comparison of reciprocal hybrids of the same age, grown under identical green house conditions. (a) A. novae-angliae  $\mathcal{Q} \times A$ . multiflorus  $\mathcal{Q}$  and (b) A. multiflorus  $\mathcal{Q} \times A$ . novae-angliae  $\mathcal{J}$ .

in sizes of the reciprocal hybrid seedlings (as measured by size of cotyledons) are correlated with differences in the sizes of the achenes (fig. 1b) in inbred A. novae-angliae and A. multiflorus, in A. novae-angliae \( \frac{2}{3} \text{ A. multiflorus } \frac{2}{3} \), and in the reciprocal cross. The different sizes of the two achenes producing the reciprocal hybrids seem to be correlated with the size of the achene in the maternal parent (Delisle, 1938). It was also noticed that the smaller achenes in these two inbred species tended to produce smaller seedlings. As was found in rice by Ramaswami (1935), these early differences gradually even out as the plants get older. The variability of the mature plants of the F<sub>1</sub> generation is relatively slight.

Plants of the  $F_2$  generation show greater variability (fig. 4) than those of the  $F_1$  hybrids. The majority of these  $F_2$  plants, however, show a tendency to approximate in morphological characters the appearance of A. novae-angliae rather than that of A. multiflerus.

The synthetic hybrid between A. novae-angliae ? and A. multiflorus of here reported is identical with A. amethystinus Nutt (of Gray's Manual). Ever

<sup>5</sup> It is to be noted that the cotyledons in this hybrid were consistently larger than those of even the maternal parent.

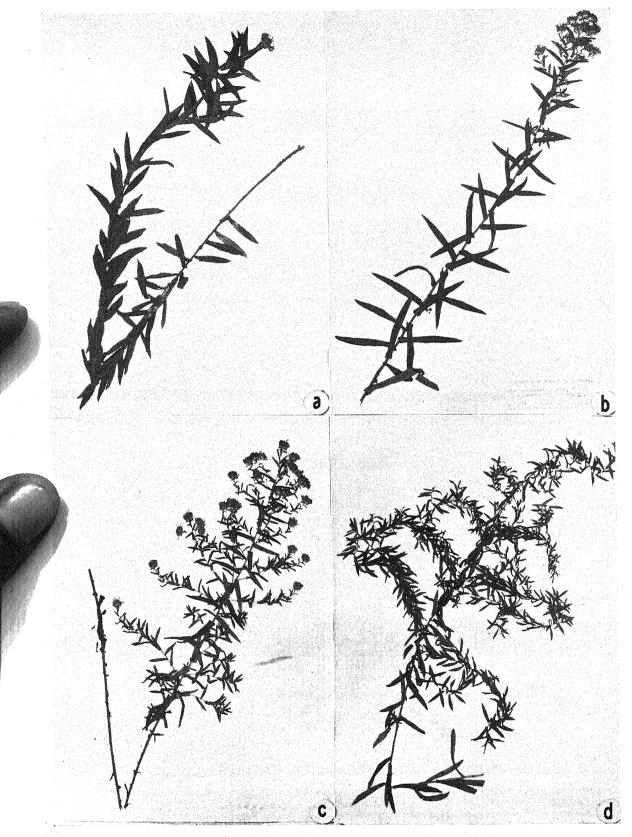


Fig. 4. Phenotypes of the F2 generation. Note variability.

Table 1. Comparison of some vegetative characters in Aster novae-angliae, A. multiflorus, and their hybrids.

Characters	A. novae-angliae	A. multiflorus	F <sub>1</sub> hybrid
Heads	Single, loose, large, in corymbs.	Very crowded, small, race-mose.	Not crowded, intermediate, loosely racemose.
Involucre	Imbricated, viscid-glandular, hairy.	Imbricated, outer part bristly ciliate, not glandular.	Imbricated, hairy, not glandular.
Involucral bracts	Herbaceous, almost equal, erect, linear, awl-shaped, loose with erect tips, 5.4-7.1 mm.;	Herbaceous tips squarrose or spreading, 2.7-2.9 mm., gradually passing into leaves on	Herbaceous tips slightly squarrose or recurved or spreading, 3.6-4.0 mm., generally abrupt
	abruptly distinct from leaves on branchlets bearing the heads.	branchlets bearing the heads.	transition into leaves on branch- lets bearing the heads.
Corolla			
Ray florets			
Length and			
breadth	$20-22 \times 1.2-1.7$ mm.	$4.0-4.4 \times 0.65$ mm.	$7.2-7.8 \times 1.0-1.1 \text{ mm}.$
Number	65 (50-68)	13	30-33
Disc florets			
Length and			
breadth	$6.6-7.1 \times 0.6-0.8 \text{ mm}.$	$3.5 \times 0.6$ mm.	$4.5-5.7 \times 0.65-0.75 \text{ mm}.$
Number	77–85	9–10	30-32
Style length	5.5–7.1 mm.	4.0–5.0 mm.	5.0–6.0 mm.
Achenes	Flattened, weakly-ribbed,	Flattened, ribbed, less pubes-	Flattened, weakly-ribbed, pu-
Form (fig. 1b)	strongly pubescent with long hairs.	cent with short hairs.	bescence intermediate.
Length Breadth Weight	2.5 mm. (2.4–2.6) 0.77 mm. (0.7–0.9) 0.43–0.45 mg.	1.5 mm. (1.3–1.6) 0.60 mm. (0.58–0.61) 0.20 mg.	2.15 mm. (2.0-2.3) 0.52 mm. (0.50-0.54) 0.30-0.40 mg.
Cotyledons (mature)			
Length	5.8 mm. (5.0-6.1)	3.7 mm. (3.6-4.1)	4.2 mm. (3.7-5.0)
Leaves Shape	Entire, cordate-clasping auriculate base, acute, soft, lanceolate, large.	Entire, scarcely narrowed at sessile base, rigid, small.	Entire, cordate-clasping auricu- late, not rigid, intermediate.
Length (mature stem leaves)	10.0–13.0 cm.	5.0–7.0 cm.	7.0–8.5 cm.
Stem	Stout, glandular-pubescent, especially above, less glandular below; high (0.5-2.5 m.).	Pale or hoary, slender with minute, close to hirsute pubescence. Usually low (0.3-1.0 m.).	Intermediate in habit, puberu- lent, slightly glandular, inter- mediate in height (0.45-1.7 m.).
Degree of branching	Unbranched when young, be- coming corymbed at summit later, upright.	Very much branched and bushy.	Upright, intermediate in branching, not bushy.
Branchlets	Viscid-glandular-pubescent, non-wiry.	Conspicuously pubescent with appressed hairs, pale or hoary, wiry.	Cinereous-pubescent, with spreading, non-glandular hairs, non-wiry.
Rhizome	Perennial and spreading.	Perennial and spreading.	Perennial and spreading.
Habitat	Moist, chiefly calcareous grounds.	Dry, sandy soil.	Moist ground.
Time of flowering	August-October.	August-October.	(August) September-October.

<sup>\*</sup> All measurements except height of plants based on data obtained from samples of 100 or more.

since A. amethystinus was first described in 1840 by Thomas Nuttall, reports of its sporadic occurrence have been presented. Since it is repeatedly mentioned that, when found, it grows close to A. novae-angliae and A. multiflorus, it has generally

<sup>6</sup> There are at the Gray Herbarium approximately twenty-five specimens and at the New England Botanical Club Herbarium thirteen specimens of this hybrid. Figure 5 indicates the distribution of A. novae-angliae, A. multiflorus, and A. amethystinus in the United States and adjacent Canada based on material available at the Gray Herbarium.

been suspected that A. amethystinus represented a natural cross between these species (Benke, 1930; Knowlton, 1930). Figure 5 shows that in all cases A. amethystinus occurs where the ranges of A. novae-angliae and A. multiflorus overlap.

Although hybridism has long been supposed, as far as the writers know, no positive proof through breeding experiment has been published as yet concerning the parentage of this species.<sup>7</sup>

 $^7$  In a personal letter to the senior author, Prof. A. J. Eames reported having pollinated plants of A. multi-

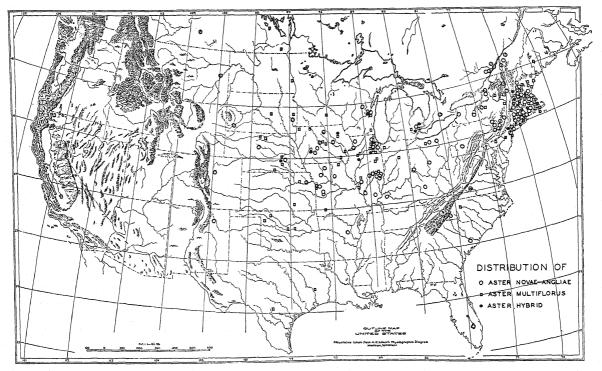


Fig. 5. Comparative distribution of A. novae-angliae, A. multiflorus, and their F<sub>1</sub>, A. amethystinus, in the United States. (Gray Herbarium Collection.)

In the fall of 1933 the writers collected a specimen with rose rays which was identified as A. amethystinus. In this case again the plant grew among many plants of A. multiflorus and within a short distance of a few plants of A. novae-angliae. It is of interest to point out that this A. amethystinus-like florus with pollen from A. novae-angliae and obtaining seed which germinated into the typical A. amethystinus. It is interesting to note that Eames seems to have had no serious difficulty in germinating seeds of this cross nor in having them flower the first year. This is in striking contrast to the results of the present work.

<sup>8</sup>This plant was found at Gerry's Landing on the Charles River, in Cambridge, Massachusetts. The speci-

men is now at the Gray Herbarium.

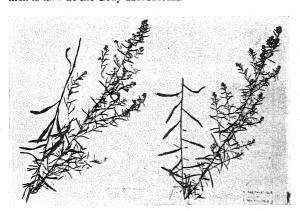


Fig. 6. Comparison of synthetic backcross of  $F_1 \circlearrowleft \times A$ . multiflorus Q (right) and specimen found in nature (left).

plant collected in Cambridge, Massachusetts, was seemingly resynthesized in the greenhouses simply by back-crossing the synthetic  $F_1$  hybrid with A. multiflorus (fig. 6, right). The resulting plant checked in all morphological characters with the specimen found in nature, as shown in figure 6 (left), and provisionally identified as A. amethystinus.

A study of these two species and of A. amethystinus in nature shows a great variability of forms. A graded series could almost be provided from one parent, through the hybrid, to the other parent. A study of herbarium material indicates the same polymorphy, apparently perplexing to the taxonomist, for forms belonging to this complex have been given different names. In breeding experiments, the writers found it possible to duplicate this range of perplexing forms, producing experimentally replicas of many of the problem plants of the taxonomist. A large number of these plants proved to be back-crosses here just as they did in Tradescantia and Iris, extensively studied by Anderson (1936), and in Solidago (Goodwin, 1937).

In order to compare laboratory material with field material, one usually makes use of qualitative taxonomic characters. A method devised by Anderson (1936) permits one to translate these qualitative characters into numerical values. Frequency distributions can then be made of whole populations on a sliding scale whose ends represent the two parent species and whose center, the hybrid. Thus when carefully selected morphological characters are to the selected morphological characters.

Table 2. Index characters used in scoring plants.

Characters	Aster novae-angliae	Index value		Index value
Diameter of fully-opened				
head	Large heads (28 mm.			
	or more)	2	Small heads (10 mm. or less)	0
Head distribution	Heads solitary	2	Heads clustered	0
Branch frequency	Paucity of branches	3	Many lateral branches	0
Leaf habit	Auriculate	3	Non-auriculate, sessile	0
Leaf and bract	Morphologically distinct	t 2	Morphologically not dis- tinct, one grading into	
			the other	0
Flower color	Rose (or blue formsa)	2	White	0
	Total index values	14	100	0

<sup>&</sup>lt;sup>a</sup> The white form is so rare as to be insignificant or negligible in this study.

acters are given numerical values, it is possible to assign each individual plant a position in the scale. Table 2 lists six contrasting morphological characters given weighted arbitrary values and chosen not only because they are of significant nature, but also because of the ease in scoring. In figures 7 and 8 appear histograms indicating the results of this method of scoring when applied to controlled genetical crosses and to field and herbarium material. The index values are plotted on a basal scale ranging from 0 (plants possessing all the characters of pure A. multiflorus) to 14 (plants possessing all the characters of the species A. novae-angliae).

These histograms show that many individuals are found in nature which are neither hybrids nor pure species. They possess morphological characters ranging from those of the hybrid to those of either parent. They are usually the perplexing forms, which have even been assigned varietal rank. The majority of these, it seems, are really the product of promiscuous back-crossing of hybrids with the

parental species. It should be mentioned that an attempted analysis of the individual characters upon which these histograms are based gives no indication of simple Mendelian segregations. Rather the ratios are complex and not at present understood. Nor are there evidences of linkage in the six characters involved. Further work must be done before any understanding of the genetic relations of these characters can be admitted.

Both A. novae-angliae and A. multiflorus prove to have 5 chromosomes as a haploid count. This number was obtained from an investigation of the pollen mother cells (Pl. 1, fig. 1, 4). Many somatic counts of 10 were seen in the nucellus, in the integuments of the ovule and in the staminal tissues of each species (Pl. 1, fig. 3). These findings confirm Carano's (1921) report of 5 chromosomes for the embryo sac of A. novae-angliae.

In A. novae-angliae there is considerable difference in chromosome size, the size-ratio of the largest to the smallest chromosome at metaphase in the

Table 3. A comparison of percentages of good pollen in Aster crosses.

Pe	Percentage Number good of loculi —			Biva	lents		Number of cells	Percent- age irregu-	
Name	pollen	counted		5	4	3	2	counted	larity
A. novae-angliae	90	22		ıll			_	100	0
$F_1 \preceq \times A$ . novae-angliae $\circ$	60-75	42		27	8	-		35	23
$F_1 \preceq \times A$ . multiflorus $Q$	30-50	20		_		-			
A. amethystinus-like plant (a									
natural backcross?)	50	8		7	6	1		14	50
A. multiflorus	85	21		all	-		-	100	0
F <sub>2</sub>	30-60	33		17	9	2	-	28	40
$F_1 \Omega \times A$ . multiflorus $\mathcal{E}$	50-54	20		19	11	2		32	40
A. multiflorus $\mathcal{Q} \times \mathcal{F}_1 \mathcal{J} \dots$	50-59	4						<del>-</del>	_
A. amethystinus-like plant ♀									
× A. multiflorus &	75	23			-	-	-	·	-
A. novae-angliae $\mathcal{Q} \times \mathcal{F}_1 \mathcal{J} \dots$	70	30		-		'	_	_	-
A. novae-angliae $\mathcal{L} \times A$ . ame-									
thystinus-like plant &	68	4		50	22	7	1	80	37

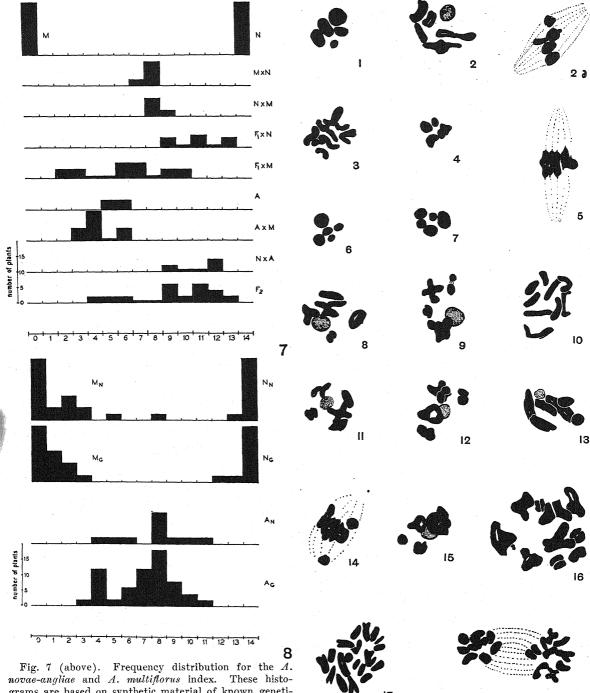


Fig. 7 (above). Frequency distribution for the A. novae-angliae and A. multiflorus index. These histograms are based on synthetic material of known genetical background (except A). Index values are shown on the scale at base: M represents A. multiflorus; N, A. novae-angliae;  $N \times M$  and  $M \times N$ , the hybrid  $(F_1)$ ; A, amethystinus-like plant found in nature.

Fig. 8 (below). Frequency distribution of A. novae-angliae, A. multiflorus, and A. amethystinus. Index values are shown on scale at base of the histograms.  $M_n$  and  $N_n$  represent data from material of A. multiflorus and A. novae-angliae in the New England Botanical Club Collection;  $M_g$  and  $N_g$ , data from similar material in the Gray Herbarium. Note the recognizable polymorphy in all material represented.

Plate 1. All figures represent a magnification  $\times 3253$ .— Fig. 1. A. novae-angliae, metaphase showing five pairs of chromosomes.—Fig. 2. A. novae-angliae, diakinesis, and metaphase (2a).—Fig. 3. A. multiflorus, somatic division in nucellus, 2n=10.—Fig. 4. A. multiflorus, metaphase, n=5.—Fig. 5. A. multiflorus, metaphase, equatorial view.—Fig. 6. A. multiflorus, forma depressa, metaphase, n=5.—Fig. 7. Reductional metaphase in F<sub>1</sub>.—Fig. 8. Diakinesis in F<sub>1</sub>.—Fig. 9. Diakinesis in F<sub>1</sub>. Note presence of univalents and bivalents and inequality of homologues in two smaller pairs of bivalents.—Fig. 10. Somatic

first meiotic division being approximately 3.5:1. There appear three large chromosomes, one medium-sized and one very small. Correspondingly, dividing somatic cells exhibit six large, two medium-sized, and two small chromosomes. The first meiotic division proceeds with much regularity as is indicated in the illustrations of prophase (pl. 1, fig. 2) and metaphase (pl. 1, fig. 2a). It is always possible to distinguish the smallest and largest pairs by their size. The medium-sized chromosome pairs can usually be so identified.

A cytological investigation of the forms roseus and geneseensis of this species has shown no detectable difference in chromosome number, size, and morphology from the condition existing in the type, A. novae-angliae. In meiosis all have been found to possess five pairs of chromosomes which behave regularly, produce viable pollen and abundant seed. The diploid (2N) number of chromosomes in dividing cells of the nucellar tissue is again found to be ten.

A study of meiosis in the P.M.C.'s of A. multiflorus has indicated much regularity in the behavior
of all five pairs of chromosomes. Figures 4 and 5
(pl. 1) show two views of a first division metaphase
plate with five pairs of chromosomes visible at the
equator. Studies in chromosome morphology in this
species indicate differences in the sizes of the chromosomes in this species. There is one large chromosome, two medium-sized, and two smaller ones, with
an approximate size ratio of 2.5:1 between the
largest and the smallest in metaphase view (pl. 1,
fig. 4). All chromosomes are considerably smaller
than those of the corresponding size class in A.
novae-angliae.

A sea-side form of A. multiflorus marked by a distinct prostrate habit was examined cytologically and the genoms found to be indistinguishable from the type with five pairs of chromosomes as shown in figure 6.

As previously indicated, crosses involving the two species are effected with ease. An outstanding characteristic of the  $F_1$  hybrids of A. novae-angliae  $\mathcal{P} \times A$ . multiflorus  $\mathcal{F}$  is their comparatively high fertility. As a result there is no difficulty in obtaining plants of the  $F_2$  generation. Figure 7

<sup>9</sup> This plant, collected at Montauk, Long Island, New York, because of its extreme prostrate habit, has been transferred to and grown under greenhouse conditions for more than two years without any noticeable change in growth-form. Such a comparable coastal ecotype has been described by Turesson (1922) in Atriplex.

metaphase in nucellar tissue of reciprocal hybrid (A. novae-angliae 3).—Fig. 11. Diakinesis in F2.—Fig. 12. Diakinesis in F1  $\mathcal{Q} \times A$ . novae-angliae 3.—Fig. 13. Diakinesis in F1  $\mathcal{Q} \times A$ . multiflorus 3.—Fig. 14. Metaphase in F1  $\mathcal{Q} \times A$ . multiflorus 3.—Fig. 15. Diakinesis in A. amethystinus-like plant.—Fig. 16. A. novae-angliae, P.M.C. showing ten pairs of chromosomes.—Fig. 17. A. novae-angliae, somatic cell from nucellus showing twenty chromosomes.—Fig. 18. A. novae-angliae, anaphase, first division of the 3 gametophyte, showing ten chromosomes in each daughter cell.

(pl. 1), shows a reductional metaphase of the F<sub>1</sub> hybrid and indicates comparative sizes of the chromosomes with respect to the parental genoms (figs. 1, 4). It is to be noted that in homologous pairs the larger chromosome from the A. novae-angliae parent can usually be distinguished at diakinesis (pl. 1, fig. 9). It is interesting also that the total diameter of the plate at metaphase is intermediate between those of corresponding plates in the parental species. Figure 8 is that of a diakinesis with five pairs of chromosomes, by far the most frequent type; figure 9 shows a diakinesis with four bivalents and two univalents. The latter occurs in approximately 23 per cent of the cells examined. The rest are apparently normal.

Morphologically these plants<sup>10</sup> are intermediate in character between their two parents. The affinity between the chromosomes of the two species is fairly strong in the four larger pairs of chromosomes and weakest in the smallest unequal pair, the members of which tend occasionally to dissociate or fail to pair altogether.

The abundance of plants of the  $F_2$  generation has provided ample material for a study of the meiotic process in these plants. A selection of buds from distinct phenotypes (fig. 4) in the  $F_2$  generation has indicated appreciable pollen sterility and some irregularity in meiosis. Figure 11 (pl. 1) indicates four bivalents and two univalents in one of the diakineses, suggesting a premature separation of partners or absence of pairing due to a lack of complete homology between the partners. As shown in figure 4, the variability of these  $F_2$  plants is greater than that of the  $F_1$ .

Figures 12 to 14 (pl. 1) represent diakineses and metaphase plates in the reciprocal back-crosses of the  $F_1$  plants with the parents. Table 3 indicates approximately the relative amount of pollen sterility<sup>11</sup> and the frequency of bivalents and univalents in these back-crosses. Figure 15 shows a diakinesis in a plant collected in the field and suspected of being a natural back-cross of an  $F_1$  plant with A. multiflorus. From breeding experiments this plant has been shown (fig. 9) to be a natural back-cross of an  $F_1$  hybrid with A. multiflorus.

The two species of Aster, A. novae-angliae and A. multiflorus and their forms and A. subulatus (Wetmore, in ed.) are the only Asters as yet reported possessing five pairs of chromosomes. This number is the lowest in a genus whose species preponderantly contain nine pairs of chromosomes or multiples of this number (Tahara and Shimotomai, 1926; Wetmore, in ed.). The chromosomes in the other reported species of the genus also show wide variation in size (Tahara and Shimotomai, 1926),

 $^{10}$  Due to the retarded condition of the reciprocal hybrids resulting from the cross A. multiflorus  $\mathbb{Q}\times A.$  novae-angliae  $\mathcal{E}$  and the relative scarcity of bud material, only a few plants have been brought to flower. Figure 10 shows a somatic mitosis (2N=10) in the nucellar tissue of the hybrid.

11 Those pollen grains of much smaller size than normal, with crushed walls and disorganized contents, were arbitrarily selected as being abnormal and sterile.

reaching their largest size in Aster Tripolium. There is also considerable range in chromosome size within the genom of each individual species.

During the investigation on chromosome behavior in Aster novae-angliae, one plant was found possessing tetraploid P.M.C.'s containing ten pairs of chromosomes (double the usual number), as shown in figure 16, in association with others with five pairs in the same loculus. Observations of dividing somatic nuclei in the nucellar and staminal tissues of this plant showed the occasional presence of giant tetraploid cells containing twenty somatic chromosomes (fig. 17). These are scattered heteroploid cells with the aberrant number of chromosomes and are not chromosomal chimeras of the sectorial or periclinal type (Ruttle, 1927). Figure 18 shows the first division of the gametophyte in a heteroploid microspore. It is not yet known how this tetraploidy came about in this plantwhether by temperature changes or by other means -(Peto, 1935, 1936; Sax, 1937). The capitula in which these heteroploid cells appeared were of larger size than those of the normal diploid plants of the same species. The plants, however, were not of larger size and showed no other external difference. A comparison of the cells of the leaf epidermis showed no difference in size from that of the normal diploid A. novae-angliae. It is of interest to note that somatic counts from root-tip smears gave only diploid numbers.

Discussion.—Breeding experiments in the production of controlled genetical crosses of two species of Aster have provided specimens which make possible the analysis of field and herbarium material. Thus, a single collection of an obvious intermediate plant (fig. 6) has been shown by means of breeding and by quantitative indices to be a natural backcross of the hybrid with one of its parents, A. multiflorus. This presupposes the existence of the natural hybrid at that locality at some earlier date and its subsequent back-crossing with A. multiflorus.

Distinct varietal hybrids can be produced from the crosses between the varieties of A. novae-angliae and A. multiflorus. The hybrid resulting from the form roseus of A. novae-angliae and A. multiflorus has rose-rayed heads and is otherwise indistinguishable from the blue-violet-rayed hybrids; it is similar in all respects to the spontaneous A. amethystinus f. leucerythros n. f. ligulis roseis (Bemis, 1930).

The parallel occurrence of five chromosome pairs in both A. novae-angliae and A. multiflorus, the general similarity of the genoms, and the degree of homology in their synaptic associations when these two species are crossed establishes a close cytological relationship between them. This is confirmed by the abundance of viable pollen and good seed produced in the  $F_1$  plants. In view of the general existence of five chromosomes (haploid) in these Asters and in view of the prevalence of higher chromosome numbers, 9, 18, 27, in other species of the genus, it is suggested that there are two chromosome series, a 5- and a 9-series, with 5 as a pos-

sible basic number in the genus. This difference in chromosome number therefore might well act as a genetic barrier to the production of viable hybrids between either of these two species of Aster with five pairs of chromosomes and any other species of the genus with nine or more pairs of chromosomes. No such hybrids are known to the authors. However, direct experimental testing of this suggestion has not yet been carried out.

It is of interest to note that five pairs of chromosomes constitute a basic number for Crepis (Hollingshead and Babcock, 1930). Lactuca exhibits an interesting similarity in chromosome number with the genus Aster, ranging from 5 in L. lanceolata (Tahara and Ishikawa, 1911), 9 in L. sativa (Gates and Rees, 1921) to 11-12 in L. Thunbergiana (Tahara and Ishikawa, 1911). Some other Compositae with five chromosomes are Picris hieracioides (Ishikawa, 1911), Melitella pusilla (Chiarugi, 1927), and Hypochaeris maculata (Tischler, 1928). Babcock and Cameron (1934) have shown that chromosome number and chromosome morphology are valuable taxonomic criteria in the genus Crepis. They concluded that the primary evolutionary process which has operated in the history of the genus has effected a transformation of 6- and 8-chromosome types from 10-chromosome ancestors. Other changes have come about by interspecific hybridization and amphidiploidy, polyploidy, and a concurrence of gene mutations. It is not yet known to what extent these phenomena have operated to produce the types with large chromosome number in the genus Aster. Further studies are intended in these directions.

Though much of the polymorphy in these species of Aster is referable to crossing in these plants, the possibility is not precluded that variation of a true hereditary nature within the species does occur, as was pointed out by Gregor et al. (1936) as a result of a study of experimental taxonomy in Plantago maritima. The color varieties of A. novae-angliae are examples. Since these two species are continuously cross-pollinated (being self-sterile), as is Aster anomalus described by Anderson (1928), there is reason to expect that some variation will appear in the progeny of such outbred plants. Those species which are continuously cross-pollinated will form new combinations in every generation, and the reappearance of distinctive combinations of characters from one generation to the next will tend to be a corollary of the degree of inbreeding. Turesson (1922) assigns the term "ecotype" to the "product arising as a result of genotypical response of an ecospecies to a particular habitat." It may well be that A. multiflorus is an ecospecies possessing ecotypes, such as the seaside prostrate form previously mentioned.

Genetical and field studies along with cytological investigations have shown the common occurrence in nature of hybrids such as A. amethystinus or such as Pentstemon neotaricus (Clausen, 1933). Clausen's studies, however, show this latter species

to be a natural true-breeding, amphidiploid hybrid between P. laetus and P. azureus. It is obvious that genetical experimentation aids in recognizing spontaneous hybrids in nature and opens up possibilities for the application of the experimental method in taxonomy (Müntzing, Tedin, and Turesson, 1931). The study of artificial or spontaneous hybrids between species or between biotypes within a species gives background for the interpretation of potential as well as actual polymorphy. It helps especially to understand why certain morphological characters are correlated in nature, through linkage or pleiotropy such as was shown by Clausen in Viola (1926) or through selective correlation as demonstrated by Tedin in Camelina (1925). Thus the puzzling variants between these two species of Aster, which present the double problem of origin and taxonomic classification can be relegated to the position of back-crosses or secondary hybrids one or more generations removed from the original cross between the species.

### SUMMARY

Genetical and cytological investigations in two species, forms, and their reciprocal hybrids in Aster afford an explanation for the polymorphic complex comprised of A. multiflorus Ait., A. novaeangliae L., and the natural gradations between them. Breeding experiments have provided plants which typify the polymorphy exhibited by this group in the field.

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Utilizing a method by which qualitative characters are converted into quantitative numerical values, there was obtained confirmation of the genetical findings that these two species and their varieties hybridize extensively in nature. By this means there is produced a series of variable forms ranging in morphological and cytological characters from true hybrids, through back-crosses, to the type species. Thus there results a perplexing polymorphy, and forms belonging to the complex have been given different names by taxonomists.

Cytological investigations have indicated these two species of Aster possess a haploid chromosome complement of n = 5. A plausible explanation for the readiness of hybridization between these two species is found in the presence of similar genoms, each with five pairs of chromosomes. In the hybrid, these chromosomes exhibit remarkable affinity in the meiotic process and suggest a close phylogenetic relationship between the parents.

There are two chromosome groups in the Asters so far studied, a 5- and a 9-, with a polyploid series in the latter. The differences in chromosome number between these two groups, therefore, may act as a genetic barrier to the production of viable hybrids between the crossing of a species with five pairs of chromosomes and another species of the genus with nine or more pairs of chromosomes.

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# THE EFFECT OF DROPS OF WATER ON LEAF TEMPERATURES 1

# Paul J. Kramer

Numerous investigations have been made of the temperature of leaves in relation to insolation, applications of various spray materials, the cooling effects of transpiration, and other factors. It appears, however, that no one has investigated the effects of drops or films of water on leaf temperatures. This is particularly surprising in view of the popular belief that leaves covered with drops of water can be seriously injured if exposed to the hot sun. Two explanations of such injury are commonly offered. One explanation is that drops of water on the leaves act as lenses, concentrating the sun's rays on the leaves and overheating the tissue. The other explanation is that the films and drops of water on the leaves decrease or stop transpiration, and the leaves, deprived of its cooling effect, become too hot. Such injury is supposed to occur when the sun shines brightly immediately after a summer shower or when plants exposed to the sun are sprinkled during the middle of the day.

Injury caused by sprinkling plants which are exposed to the hot sun apparently is much less common than ordinarily is supposed. Several botanists have told the writer that they believe such injury occurs, but considerable inquiry has failed to reveal specific examples known to have been caused in this manner. Although our botanical greenhouses, containing a wide variety of plants, are sprinkled regularly in the hottest part of the day during the summer, no damage has ever resulted. It seemed worth while, therefore, to determine the actual effect of drops and films of water The plants used were on leaf temperatures. African violet (Saintpaulia ionantha Wendl.), bryophyllum, camellia, geranium (Pelargonium), sedum, and yellow poplar. All plants were growing in pots except the camellia, which was growing in soil outside a laboratory window.

The leaf temperatures were measured by means of copper-constantan thermocouples connected through switches to a potentiometer and galvanom-

<sup>1</sup> Received for publication November 9, 1938.

eter. A junction was inserted in each side of a leaf blade and brought into close contact with the lower surface. The sedum leaves were thick enough to permit insertion of the thermocouples into the mesophyll tissue. Because of the small size of the leaves, the two thermocouples were inserted into separate leaves of sedum and African violet. In some experiments the plants were exposed to the sun, but a number of experiments were performed in the laboratory under a 500 watt mazda lamp equipped with a reflector. Variations in light intensity and especially in air movement caused rapid fluctuations in temperature out-of-doors. The procedure and the results were similar in the two experiments. After readings had been made with both junctions to determine the temperatures of the dry leaves, water was sprayed from an atomizer on the surface above one junction while the surface above the other junction was left dry. Readings were then made at intervals to determine the temperatures of the wet and dry portions of the leaf or, in the case of African violet and sedum, of the wet and dry leaves. In some experiments the water was blotted up and the leaf dried with filter paper or the water allowed to evaporate and the temperature again determined. In certain experiments the wet and dry sides were reversed to neutralize any differences in contact of the thermocouples with the leaves.

The results of several typical determinations are shown graphically in figure 1. In every instance, the presence of water on a leaf or portion of a leaf was accompanied by a reduction in temperature. The reduction varied from 4 to 12°C., the average reduction for all determinations being approximately 8.5°C. The amount of reduction in temperature seemed to vary with the leaf and air temperatures and with the humidity of the air. Since this study was concerned primarily with the effects of water drops on leaf temperatures, no extensive comparison of leaf and air temperatures was made. Air temperatures were determined by

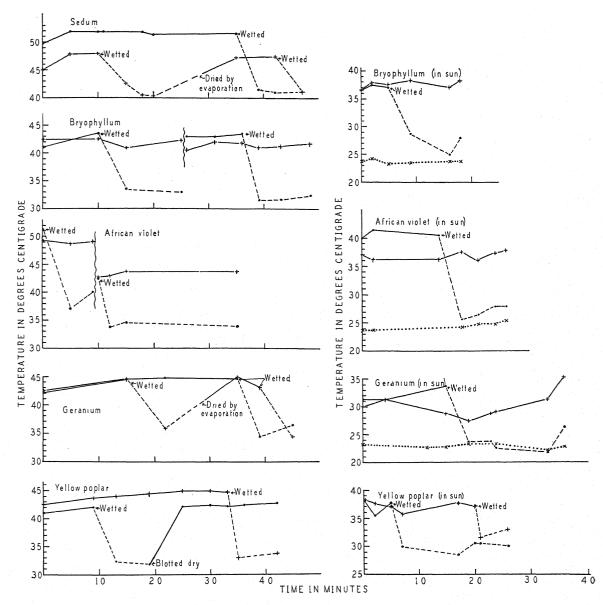


Fig. 1. Effect on leaf temperatures of wetting leaves. Each pair of lines represents simultaneous temperature determinations on a leaf or pair of leaves. Solid lines represent temperatures of dry leaves, broken lines represent temperatures of wet leaves. The dotted lines on the three graphs for species in the sun are air temperatures as determined by a shaded thermometer. The vertical lines on the graphs of African violet and bryophyllum separate determinations made on different leaves.

a shaded mercury thermometer in some experiments out-of-doors, however, and are shown by dotted lines on three graphs. It appears that the temperature of the wet leaves fell approximately to that of the air.

No difference was found between the behavior of hairy leaves, such as those of geranium and African violet, and smooth, highly cutinized leaves such as those of bryophyllum, camellia, and yellow poplar. The thick, succulent leaves of sedum behaved in the same manner as the thinner leaves of the other species. Water sprayed on the hairy

leaves tended to coalesce into films, but on the cutinized leaves it accumulated in drops. In some of the earlier experiments a single drop of water was placed on the leaf surface above the thermocouple junction, and the cooling effect was just as pronounced as when a larger area was wetted.

The results of these experiments are in accord with what would be expected on theoretical grounds. About 574 calories is required to evaporate one gram of water at 40°C. The removal of such a large quantity of heat would naturally materially decrease the temperature of the adjacent leaf tis-

sue. This would certainly more than compensate for any decrease in the cooling effect of transpiration.

Those who have suggested that drops of water on the leaves might act as lenses, concentrating the sun's rays and so burning the leaves, apparently have never calculated the focal length of such lenses. Observation of leaves after sprinkling indicated that drops from one to five millimeters in diameter are commonly present. Larger drops are usually irregular in outline, having resulted from

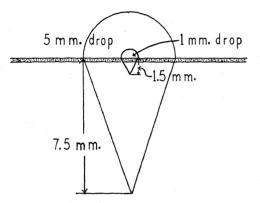


Fig. 2. Diagram showing focal distance of water drops acting as lenses on a leaf 0.5 mm. in thickness. Many leaves of course are much thinner.

the coalescence of several smaller drops. Very small drops evaporate so quickly in the bright sun that they could scarcely cause injury. Assuming that the average drop may be treated as a planoconvex lens, the focal length of a drop one millimeter in diameter is 1.5 millimeters or many times the thickness of the average leaf.2 Larger drops have correspondingly greater focal lengths. Large drops form somewhat flattened lenses which further increases their focal length. An attempt was made to measure the temperature by focussing on the junction of a thermocouple the visible rays from drops of water placed on a glass plate under a bright light. This was done for several drops of water, but no significant increase in temperature was observed. Considerable infra-red radiation is doubtless absorbed by the water and apparently very little reaches the focal point. Injury to leaves from a lens action of water drops on their surfaces is certainly rare and probably never occurs.

<sup>2</sup> The writer gratefully acknowledges the assistance of Prof. C. C. Hatley of the Physics Department in calculating the focal length of the water drops, and the aid of T. H. Wetmore and J. J. McDermott in making the determinations of leaf temperature.

It would seem probable theoretically that the presence of water on leaves might reduce rather than increase injury from overheating. This was tested by placing plants of several species so that their leaves were only a few inches from a 500 watt mazda lamp equipped with a reflector. Leaf temperatures of 50 to 60°C. were easily obtained, and leaves exposed to the light were killed in five to ten minutes. It was found, however, that leaves which were kept covered with a film of water applied from an atomizer remained uninjured, but that when the water was allowed to evaporate, the leaves were soon injured. When the water began to evaporate those areas which first became dry were the first to show injury, and no injury was ever observed under drops or films of water.

Watering plants during the middle of the day doubtless results in considerable wastage of water by excessive evaporation. Wetting the foliage also increases the possibility of fungus infections. It is further possible that injury may occasionally result from sprinkling leaves which bear bits of fertilizer or other foreign material. It is highly probable, however, that little or no direct injury ever results from the presence of water drops on leaves exposed to the sun.

## SUMMARY

The effect of drops of water on leaf temperatures was investigated by inserting thermocouples into leaves which were sprayed with water. thermocouples were usually inserted into a leaf, and the surface above one was kept dry while the surface above the other thermocouple was wetted. The leaves were exposed to the sun or to a 500 watt mazda lamp and the temperatures of the wet and dry portions determined. The temperature of the leaf areas covered with water drops was 4 to 12°C. lower than the temperature of the dry areas, the average reduction being 8.5°C. Wetted leaves placed a few inches from a mazda lamp remained uninjured, while dry leaves similarly exposed were killed by the heat. It was found that drops of water probably cannot cause injury by acting as lenses and concentrating the sun's rays on the leaf tissues because the focal length of such drops is usually much greater than the thickness of the leaves. It is concluded that injury to leaves from exposure to the sun while covered with water drops is much less common than popularly supposed, and possibly never occurs.

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# PHOSPHATE RETENTION BY SAND IN RELATION TO SEEDLING CULTURE 1

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PHOSPHATES ARE known to be fixed by soil particles and by sand. When complete fertilizers are applied to soil, the phosphorus is one of the last elements to be leached out by rain. Similarly, the addition of nutrient solutions to sand cultures frequently results in retention of some phosphate by the sand. Although phosphates become fixed in a form that is insoluble in water, they still may be available to plants through the activity of the roots. Among the many soil studies on phosphate retention, the work of Ford (1933), Perkins and King (1936), and Beater (1937) may be mentioned.

In regard to the sand culture of seedlings, the importance of phosphate retention became evident in connection with certain experiments for the control of damping-off. It was noted that seedlings made as good growth in sand previously used with a complete nutrient solution, washed in water and then supplied only with KNO<sub>3</sub>, as in new sand with both KNO<sub>3</sub> and Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub> supplied. Later it was found (Dunlap and Livingston, 1937) that considerable phosphate remained in the moist sand mass in a porous flower pot under continuous subirrigation, even after the K and NO<sub>3</sub> had been completely removed by evaporation and water movement.

As preliminary experiments, the growth of seedlings in new sand with only  $KNO_3$  added was compared with growth in the same kind of sand but with both  $KNO_3$  and  $Ca(H_2PO_4)_2$  supplied as plant nutrients. In this way, young plants of celery, lettuce, spinach, and tomato were found to respond markedly to the additional application of  $Ca(H_2PO_4)_2$ . On the other hand, barley, cabbage, flax, garden pea, kochia, and zinnia showed little difference in size of the seedlings when  $Ca(H_2PO_4)_2$  was included as a nutrient. In this connection the P-content of seeds is known to vary considerably between different species.

This paper reports the results of experiments on phosphate retention by sand as indicated by the early growth of plants from seed. A white, insoluble sand (Berkeley No. 18 mesh, Pennsylvania Glass Sand Corporation), practically neutral in reaction, was used as the culture medium for the plants. Over one hundred cultures of about 125 tomato seedlings each were grown in the greenhouse at the Connecticut Agricultural Experiment Station during the winter of 1937-1938. were two series of cultures. The first consisted of new sand entirely, with various nutrients [mostly KNO<sub>3</sub> with varying amounts of Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub>] added. After the plants in the first series were harvested, the same sand was washed and used over again in the second series of cultures to which only KNO3 was added in most cases. Some of the cultures in each series contained sand that had been washed in 8 per cent HCl. In addition to the green-

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house cultures, laboratory studies were made to determine the relative rapidity and degree of phosphate retention when different types of sand were treated with solutions of  $Ca(H_2PO_4)_2$ . Except for certain modifications, the method for growing the experimental plants was the same as that described by Dunlap and Livingston (1937). The weights recorded below refer to the total, fresh, stem and leaf material produced by the entire culture 28 days after sowing.

Obviously the fresh-weight data obtained from the plants in the first series of cultures was in no way related to phosphate retention by the sand, since phosphates are available even after fixation. In the first series, cultures without added nutrients and receiving only tap water produced 5.5 gm, of fresh material. Those receiving a single-salt application of 1 gm. (about 0.01 gm.-mol.) of KNO<sub>3</sub> per culture produced an average fresh weight of 21.8 gm. Different applications of Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub>, in addition to the above amount of KNO<sub>3</sub>, per culture at time of planting gave the following increases in fresh weight:

Amount of Ca(H <sub>2</sub> PO <sub>4</sub> ) <sub>2</sub>	Fresh weight of seedling						
0.000 gm.	21.8 gm.						
.004	22.2						
.02	34.2						
.1	46.3						
.5	56.5						
2.5	58.4						

It may be seen from the above figures that applications of 0.5 gm. (about 0.002 gm.-mol.) of  $Ca(H_2PO_4)_2$  in addition to 1 gm. of KNO<sub>3</sub> resulted, under these conditions, in practically the maximum amount of plant growth; and smaller quantities apparently were inadequate. When this phosphate application was increased five times [to 2.5 gm. of  $Ca(H_2PO_4)_2$  per culture], an increase in fresh weight of only about three per cent was obtained.

In the second series, only 6 gm. of fresh material were produced in the unfertilized sand. About 23.5 gm. fresh weight was recorded for those cultures that received only KNO3 for the second time in succession. The cultures that had received previously 0.5 gm. or less of Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub> produced seedlings whose total fresh weights per culture were considerably less (mostly 25 to 30 gm. total fresh weight per culture) than those obtained in the corresponding culture of the first series. There was a marked increase, however, in the fresh weights of all cultures in the second series which previously had received 2.5 gm. of Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub> as compared with the cultures which had received smaller amounts. The average fresh weight of 4 cultures that had received 2.5 gm. applications of Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub> in the first series was 49.2 gm. This figure compares rather favorably with the 58.4 gm. obtained from previous plant growth in this same sand when the phosphate had been freshly added.

Acid treatment of the sand had no marked effect upon the growth of the tomato plants in the first series of cultures, as compared with untreated sand. In the second series, however, the plants growing in sand that was washed in acid before the first sowing of seeds were considerably smaller [about 30 gm. fresh weight, in each of those cultures that received 2.5 gm. of Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub>] than those in cultures with sand that was washed only in water (about 49.2 gm. fresh weight) the first time the sand was used. This fresh weight of 30 gm. is noticeably greater, however, than the 23.5 gm. obtained in the cultures with sand that had received no phosphate in either series. Washing of the sand with acid just before replanting of the cultures resulted in uniformly poor growth in the second series (KNO3 only) regardless of the amount of Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub> that had been added in the first

In place of the Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub> used in the cultures mentioned above, some cultures of the first series were treated with KH2PO4 and others with K<sub>2</sub>HPO<sub>4</sub>, in addition to the KNO<sub>3</sub>. These two phosphates were substituted for the Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub> in a few cases because of their different solubilities and pH values. In a limited number of tests there was little difference between the fresh weights obtained from these three sources of P, in either the first or second series of cultures. This would seem to indicate that phosphate retention had taken place in this type of sand regardless of differences in the initial pH of the nutrient solution. In the cultures to which KNO3 and Ca(H2PO4)2 were added, the initial hydrogen ion concentration of the nutrient solution was around 4.0 pH. However, the solution in the sand mass at the time the cultures were discontinued (28 days after planting) was found to have a pH value of 6.5 to 7.0.

The results of laboratory studies of the physical and chemical properties of certain different kinds of sand in relation to their phosphate-retaining capacities are given in table 1. The method of Truog (1930) with certain modifications<sup>2</sup> was used in ascertaining the amount of available P which remained in the sand after treatment with 0.1 per cent solution of  $\text{Ca}(\text{H}_2\text{PO}_4)_2$  for 5 days and then carefully washed.

Untreated samples of the above grades of sand gave very low tests for P (not more than 3 ppm. in any case), and all but a trace of this was removed by the acid treatment. When these acid-washed sands were treated with the 0.1 per cent  $Ca(H_2PO_4)_2$  solution, smaller amounts (as compared with sand washed only in water) of available P were found to be retained, as is shown by the figures in the last column of table 1. Results almost identical to those in table 1 were also obtained when

Table 1. Amount of P retained by different kinds of sand.

				wash-
Туре	Color	Source	_	HCl ppm.
A	Red-brown	Inland pit near New Haven, Conn	42	19
В	Yellow	North shore of Long Island, N. Y.	27	8
C	Gray	South shore of Long Island, N. Y	18	3
D	White	Penn. Glass Sand Corp. (used in above cul-		
$\mathbf{E}$	White	tures)	8	2
		same particle size as above sands)	1.6	1,

 ${\rm KH_2PO_4}$  was used in place of the  ${\rm Ca(H_2PO_4)_2}$ . When particles of the A type sand were separated according to size, the following data as to P retention was obtained: fine particles (0.3 mm. or less in diameter), 70 ppm.; medium particles (0.3 to 0.8 mm.), 38 ppm.; coarse particles (larger than 0.8 mm.), 41 ppm.

As to the rapidity of retention, untreated sand of the A type after standing in a 0.1 per cent solution of Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub> for different periods of time was found to have retained P as follows: 1 hr., 16 ppm.; 2 hrs., 19 ppm.; 4 hrs., 30 ppm.; 1 day, 31 ppm.; 4 days, 36 ppm.; 7 days, 47 ppm. With a saturated Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub> solution, 105 ppm. of P were retained in 2 hours and 280 ppm. in 7 days by this same type of sand.

Discussion.—Under the conditions of these experiments the phosphate requirements of tomato plants, weighing approximately 0.4 gm. each at an age of three weeks after emergence, were largely satisfied by phosphate retained in the sand in which the seeds were sown. The sand used in this case had a relatively low phosphate-retaining capacity as was shown by the laboratory tests. The retained phosphate had been added, as a soluble salt, in excess of plant needs to the sand at the time of planting a previous series of cultures. At least a part of this excess phosphate was not removed from the sand by several thorough washings in water; the phosphate was considered, therefore, to be retained in an insoluble state by the sand. The actual amount of phosphate potentially available to each plant would depend, obviously, upon the volume of sand within reach of the root system, and upon the amount of phosphate held in a given amount of sand. It has also been shown by the above laboratory tests that the most highly colored and finest grades of natural sand retained the most phosphate per unit weight. Screening out the finer particles or washing away the silt from the sand would tend to reduce the amount of phosphate retention. Washing of the

<sup>&</sup>lt;sup>2</sup> Tests supervised by Mr. H. G. M. Jacobson.

sand in acid also removes much of its phosphate-

retaining capacity.

From this work alone, conclusions as to the nature of the phosphate retention by sand would scarcely be justified. The fact that the results obtained with KH2PO4 were similar to those with Ca(H2PO4)2 would indicate actual fixation by the sand rather than merely mechanical retention of precipitated phosphate salts. The mere trace of P retention (1.6 ppm.) found with crushed glass of the same particle size as the sands used would also indicate that adsorption of the phosphate did not take place by this material. A chemical reaction may occur between the soluble phosphates and certain impurities of the sand. The marked retention of phosphate that was found in connection with the colored sands is in keeping with the belief that the Fe content of the sand influences the amount of fixation. Ford (1933) found that P fixed in soil as Fe and Al phosphates were at least in part easily hydrolizable and available to plants. He also showed that goethite and partially dehydrated bauxite formed phosphates that were crystalline on X-ray analysis. Such reactions between sand and a nutrient solution might alter the composition of the latter without materially changing its concentration, because of base exchange.

## SUMMARY

Young tomato plants fertilized with only KNO<sub>3</sub> in used sand, which in previous cultures had re-

ceived applications of  $\mathrm{Ca}(\mathrm{H_2PO_4})_2$  in excess of plant needs and then carefully washed, were practically equal in fresh weight to plants in new sand with both  $\mathrm{KNO_3}$  and  $\mathrm{Ca}(\mathrm{H_2PO_4})_2$  added. Treatment of the sand with acid lowered its phosphateretaining capacity as was shown by growth of the plants. The results of laboratory tests showing the amount of P retention as related to kind of sand, size of particle, acid treatment, concentration of the added  $\mathrm{Ca}(\mathrm{H_2PO_4})_2$  solution, and length of time of treatment with this solution are also given.

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# NOTES ON THE SCUTELLARIAE OF EASTERN NORTH AMERICA. I

Carl Epling

GALERICULARIA.—While Scutellaria galericulata occurs widely throughout northern North America, its immediate associates fall into two species-groups -a western one, ranging from the Rocky Mountains to the Pacific, and an eastern one, lying largely east of the Mississippi River. The western species group, which is being dealt with in a separate paper, is the more diverse in habit. From the evidence which I have reviewed, it seems to me not improbable that at least two of those species, S. californica and S. antirrhinoides, have been derived through hybridization of some such prototypes as S. Bolanderi and S. angustifolia. Whether S. galericulata has played a part still earlier in initiating this complex (including also S. Austinae, siphocampyloides, and S. Brittonii) is difficult to determine.

In the eastern species-group, however, it seems to me both suggestive and helpful to assume that S. australis, S. nervosa, S. Leonardi, and S. parvula have all been derived by segregation from a complex which was initiated sometime during or after glaciation by S. galericulata and some such prototype as S. nervosa (or indeed, perhaps S. lateriflora), brought together perhaps by the events of

<sup>1</sup> Received for publication August 9, 1938.

the glacial periods. The relationships of the other species, here associated with *S. lateriflora*, are more obscure, but, as pointed out later, some relationship with that species seems probable.

I. S. galericulata L., Sp. Pl. 599. 1753.—The name S. epilobiifolia of A. Hamilton was taken up by Fernald (Rhodora 23: 85, 1921) to designate the American homolog of the Eurasian S. galericulata L. This action was based upon presumed differences in corolla size and color, differences in degree of pubescence, the fact that the leaves of the American plants are more cordate at the base than the Eurasian, and particularly upon the differences in size and pattern of the nutlets. After examination of a very large series of specimens from most of the larger American herbaria I have found that these conclusions may be sustained only in part.

It is impossible to determine finally either from herbarium specimens or published plates the differences in color. In so far as recent and well prepared specimens from both regions indicate, the differences are not significant, and seemingly the variation in pattern is about the same in both re-

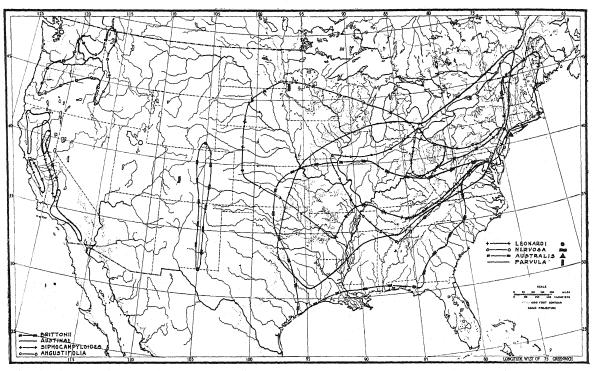


Fig. 1. A generalization from Map 2, showing the distributions in outline of the species of Galericularia. Although the relationships between the species within each group are about the same, as expressed in morphological characteristics, it will be observed that the ranges of the eastern species have become much less segregated than those of the mountainous western states. Apparent extra-limital occurrences are shown as follows: S. australis on Long Island; S. parcula in western Wisconsin; S. Brittonii in western Colorado and S. angustifolia in maritime Washington. A dubious form of the latter occurs in Utah.

gions. In degree and kind of pubescence the range of variation is about the same. Plants from Oregon and Washington may be wholly glabrous. They apparently may or may not occur with pubescent forms.

Such glabrous forms have been collected in Crook and Deschutes Counties, Oregon, and in Grant and Okanogan Counties in Washington. Apart from these wholly glabrous forms of the northwest, nearly glabrous forms are occasional in the eastern United States. Specimens from Siberia and Turkestan and occasional ones from western Europe show pubescence as dense as or denser than the specimens of the United States. The leaf-shape and habit have essentially the same range of variation on both continents. In corolla size there are certain modal differences. The range of variation in corolla length (tube plus galea, based upon boiled dissected specimens) of the comparatively few Eurasian plants which I have examined lies between 13 and 18 mm. The average is approximately 15 mm. The range of variation for the North American specimens lies between 14 and 21. The average is approximately 17.5 mm. There is also a modal difference in pattern of the nutlets, as suggested by Fernald. However, neither I, nor a colleague who kindly examined a mounted series have been able to effect a geographic segregation on this basis. In size the range of variation is essentially the same.

Since mature nutlets are generally lost in pressing, due to the habit of falling away which characterizes the upper lip of the calyx, they are difficult to study in the herbarium, and their range in variation is difficult to obtain. As far as one may judge in those instances where they have been preserved, they are frequently rather variable in pattern within a species. On the whole they apparently do not form a very trustworthy basis for specific segregation. It would appear that the deductions made by Penland (Rhodora 26: 61, 1924) were based upon an inadequate series of mature nutlets.

It is true, however, that there are racial differences between the plants of western Europe and those of Siberia and Turkestan. Furthermore, specimens indistinguishable from American and Siberian forms have been gathered in Sweden. In a few words, S. galericulata is fairly stable through most of its range in North America, save in Oregon and Washington. The extremes of the eastern United States may be duplicated in Eurasia, even in western Europe. However, the range of variation in western Europe is greater than in North America, and the extremes there apparently may not be duplicated here. In view of these facts, there appear to be, as yet, no satisfactory grounds for segregating S. epilobiifolia.

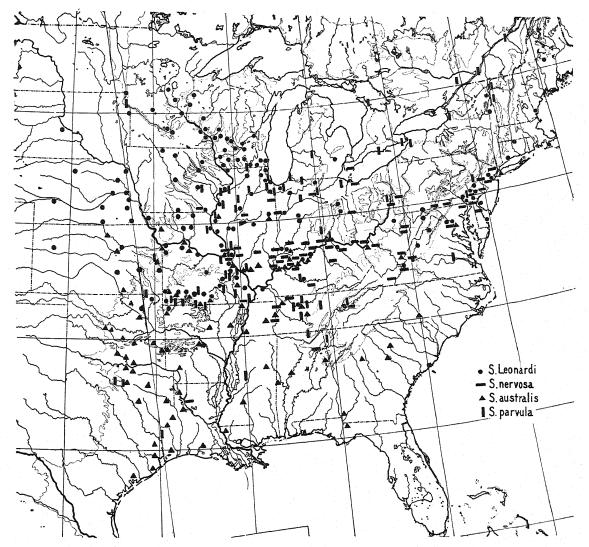


Fig. 2. A map showing the distribution in detail of Scutellaria nervosa, australis, parvula, and Leonardi.

2. S. nervosa Pursh, Fl. Amer. Sept. 412. 1814, based upon a specimen collected in Virginia; the probable type, collected at Winchester, Virginia, is in the herbarium of the Academy of Natural Sciences at Philadelphia.—S. teucrifolia J. E. Smith in Rees, Cycl. 32. no. 15. 1816, based upon the preceding.—S. gracilis Nutt., Gen. Pl. 2: 37. 1818, based upon a specimen collected by Nuttall in Pennsylvania on the banks of the Schuykill near Philadelphia; the type is in the British Museum.— S. ambigua Nutt., Gen. Pl. 2: 37. 1818, based upon a specimen collected by Nuttall in Ohio in dry and open forests; the type or a portion of it is in the herbarium of the Royal Botanic Gardens at Kew; a fragment of apparently the same plant is in the herbarium of the Philadelphia Academy; none are in the British Museum.—S. parviflora Hamilt., Monogr. 37. 1832, based upon a specimen collected by Rafinesque in Ohio; the type is in the DC. herbarium.—S. parvula var. ambigua Fernald in Rhodora 3: 201. 1901, as to name only, based upon S. ambigua Nuttall.

A perennial herb spreading by slender rhizomes, its stems now simple, now branching somewhat towards the base, sprinkled in the lowermost parts with spreading capitate-glandular hairs, in the upper parts with upwardly curving appressed hairs chiefly along the angles, sometimes wholly glabrous; blades of the lowermost leaves cordate-ovate, 1-1.5 cm. in diameter, sprinkled on both surfaces with spreading capitate glands, borne on petioles .5-2.5 cm. long, those of the median leaves generally ovate, sessile, 20-45 mm. long (averaging about 35 mm.), 12-36 mm. broad (averaging about 20 mm.), their margins unevenly crenate-dentate, those of the uppermost entire, the upper surfaces appressed hirsute, especially towards the margins, the lower hirtellous along the veins with curving hairs; flowers disposed above the middle on pedicels which are ascendent-hirtellous; lower calyx lip 2.5-3 mm.

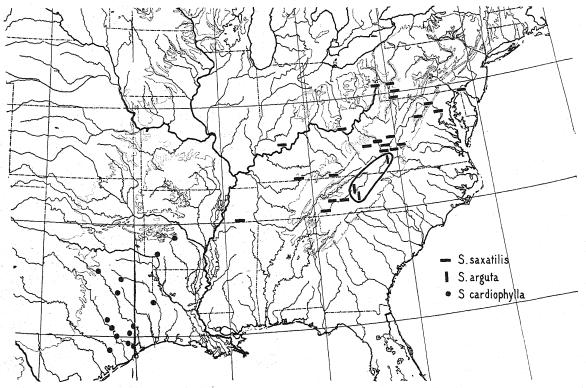


Fig. 3. A map showing the distribution of Scutellaria arguta, saxatilis, and cardiophylla.

long at flowering, 5-6 mm. long at maturity, hirtellous, the squama about 4 mm. tall, concave, galea and tube 8-10 mm. long, the lower lip hirtellous in two lines, the lower filaments seated below the middle of the tube; nutlets brown or nearly black, covered with peg-like processes, usually banded.

The principal variation of this species lies in the shape of the leaves, whether tending to rotund or deltoid. The former condition is more prevalent. Occasional specimens occur which suggest an intermixture with S. Leonardi, such as those of Short from Blue Lick, Kentucky. Perhaps such specimens, which seem largely confined to Kentucky, Indiana and Illinois are resultant from incomplete segregation of an earlier complex from which S. Leonardi has also been derived. Such specimens have little to distinguish them from S. Leonardi save in the larger parts throughout. Pursh's specimen of S. nervosa is very similar to the plants collected near Washington, D. C., such as those gathered by Shelden or Kearney. Nuttall's type of S. gracilis is fairly average. His type of S. ambigua however, which is closely matched by Lloyd's collections from Cincinnati and by those of Frank (anno 1835) from the same region, is clearly conspecific with S. nervosa and not with the plant here designated S. Leonardi. Comparison of the pubescence of this specimen with a large series of specimens including the smallest branch of the Pursh sheet and such specimens as Lloyd's, as has been done by the author, is convincing of their conspecificity. The species occurs consistently in woods in springy places or along streams.

3. S. Leonardi nom. nov. S. ambigua Leonard and other authors, not Nuttall.—S. parvula var. ambigua Fern. in Rhodora 3: 201. 1901, excluding Nuttall's specimen; Nuttall's specimens at Philadelphia labelled "Scutellaria parvula or var.," consist of S. australis and S. Leonardi. Pursh's specimens of "S. parvula" are S. Leonardi.

A small perennial herb creeping by moniliform rhizomes, its stems usually branched at the base, erect, generally 10-20, rarely 30 cm. tall, sprinkled in the lowermost parts with small spreading capitate glands, elsewhere minutely appressed-hispidulous along the angles, appearing glabrous, rarely completely so; lowermost leaf-blades rotund-ovate, usually 4-8 mm. in diameter, sparingly hirsute on the upper surfaces, borne on petioles 3-8 or rarely 11 mm. long, the median 4-6 mm. broad (averaging about 5.5 mm.), 11-16 mm. long (averaging about 13 mm.), sessile, narrowly deltoid-ovate, quite entire, rarely irregularly dentate toward the base, glabrate on both surfaces, being minutely appressedhispidulous only toward the margins and somewhat revolute; flowers disposed above the middle, the pedicels ascendent-hirtellous; lower lip of the calyx 2-3.5 mm. long at flowering, 4-5 mm. long at maturity, the squama 2-3 mm. tall, concave; galea and tube 6.5-9 mm. long, the lower lip hirtellous in two lines, the lowermost filaments seated near the middle of the tube; nutlets brown, covered with peglike processes, usually banded.

Throughout its range S. Leonardi is rather constant and may readily be recognized by the nature of its pubescence as well as by the habit and shape of its leaves. As pointed out above, it appears as a diminutive of the stricter forms of S. nervosa and frequently suggests a diminutive S. galericulata. That the two occur together in some regions seems certain. It is a pleasure to associate with this species the name of Mr. Emery C. Leonard who, in his careful and useful revision, recognized the specific distinctions clearly but, lacking access to the type at Kew, was misled as to the name.

4. **S. australis** sp. nov.—S. parvula var. australis Fassett in Rhodora 39. 378. 1937, based upon a specimen collected by Fassett (no. 18063) on sandstone ledges near White River, Goshen, Arkansas; the type is in the herbarium of the University of Wisconsin.

A small perennial herb creeping by moniliform rhizomes, its stems erect, usually branching at the base, generally 10-30 cm. tall, sparingly clothed along the angles usually with appressed-ascendent hairs and with longer spreading capitate glands, rarely eglandular; blades of the median leaves sessile, ovate and entire, rarely deltoid-ovate, and sparingly dentate, prevailingly 7-10 mm. broad, 10-15 mm. long, those of the lowermost subrotund,

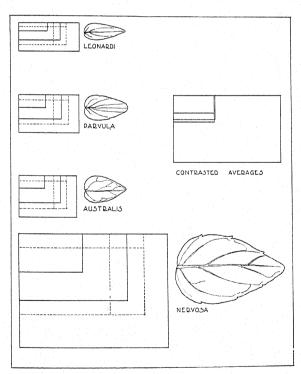


Fig. 4. A chart showing the minimum, average and maximum sizes in proportion of the median leaves (lowermost floral leaves) of Scutellaria nervosa, australis, parvula, and Leonardi. The ordinary deviations from the average are shown in broken lines. The usual shapes are shown in the outline drawings. The drawings were made from the data in table 1.

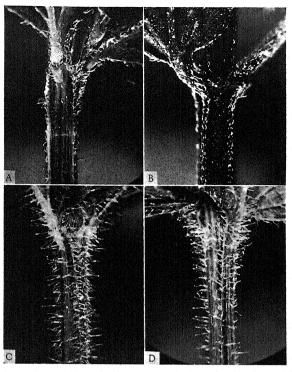


Fig. 5. Photographs illustrating the pubescence types of (a) Scutellaria nervosa, ×12; (b) S. Leonardi, ×13; (c) S. parvula, ×13; (d) S. australis, ×12.

usually 7-12 mm. in diameter and borne on petioles 5-15 mm. long, all sprinkled on both surfaces with rather long capitate glandular hairs, rarely glabrate; the veins prominent and tending to branch and anastomose along the margins; flowers disposed above the middle of the plant on pedicels which are ascendent-pubescent, but hardly appressed; lower lip of the calyx 2.5-3 mm. long at flowering, 4-5 mm. long at maturity, the squama 3.5-4.5 mm. tall, sprinkled with capitate glands, concave; galea and tube 7-10 mm. long, the lower lip hirtellous in two lines, the lower filaments seated near the middle of the tube; nutlets brown, covered with peg-like processes, usually banded.

The data concerning pubescence furnished by Professor Fassett is wholly in accord with my own observations previously made. By reason of these absolute differences, as well as the modal differences in leaf habit and the facts of distribution, it has seemed desirable to accord specific recognition to this plant. Just as S. Leonardi suggests a diminutive of the strict forms of S. nervosa, S. australis suggests in many ways a diminutive of the more average forms of that species. It will be observed that the average proportions of the leaves are almost identical. Their texture is similar.

Occasional specimens occur which appear to be intermediate with S. Leonardi, having the habit of that species but the pubescence of S. australis. Such specimens have been found at Grantville and

at Windsor Springs, Missouri. Still other specimens occur, which, while otherwise similar to S. australis, are wanting in the spreading glands. The ascendent, appressed hairs are coarser than those of S. Leonardi. Such specimens have been collected at Dillerville, Pennsylvania; Alexandria, Louisiana; Terrell, Texas; and Houston, Texas. A specimen collected by Gleason near Urbana, Illinois, was apparently cleistogamous. It, with other flowering specimens collected at Champaign by the same collector, suggest S. Leonardi in habit. A specimen collected at Fulton, Arkansas, is nearly glabrous.

point where they leave the calyx. They are accordingly erect. Those of S. cardiophylla are similar but less abruptly curved.

6. S. Churchilliana Fern. in Rhodora 4: 137. 1902, based upon a specimen collected by Fernald at Masardis in the Aroostook Valley, Maine; the

type is in the Gray Herbarium.

As suggested by Fernald and by Penland, this is not improbably of hybrid origin between S. galericulata and S. lateriflora, being about intermediate with the presumed parents. It is fairly stable. It may readily be distinguished from S. galericulata by the size of the corolla and the

Table 1. Dimensions of median leaves in mm.

	N =	max. l.	ave. l.	σ l.	min. l.	max. w.	ave. w.	σw.	min. w.
S. nervosa	73	45	33.5	5.5	20	36	21	4.5	12
S. australis	76	18	13	2	8	13	8.5	1.7	4
S. parvula	73	19	13.3	2.5	8.5	12	7.5	1.6	4
S. Leonardi	73	19	13	1.9	9	9	5.5	1.1	2.5

5. S. parvula Michx., Fl. Bor. Am. 2: 11. 1803, based upon specimens collected in Illinois and in Canada near Montreal; the type, bearing both specimens, is in the Museum of Natural History at Paris.—S. parvula var. mollis Gray, Syn. Fl. 2: 380. 1878, based upon specimens collected by Patterson at Oquawka, Illinois; the type is in the Gray Herbarium.—S. campestris Britton, Mem. Torrey Bot. Club 5: 283. 1894, based upon the preceding.

A small herb, spreading by moniliform rhizomes, its stems usually erect, branching at the base, 10-30 cm. tall, covered evenly and densely with subretrorse or spreading hairs and with larger capitate glandular ones, frequently bearing only the glandular hairs in the lower parts; median leaf-blades sessile, deltoid-ovate, rarely ovate, usually irregularly subdentate, for the most part 8-19 mm. long, 4-12 mm. broad, the lowermost subrotund, usually 7-12 mm. in diameter, borne on petioles 5-15 mm. long, the upper surfaces of all hirsute with capitate glands, the lower clothed like the stems, also more or less sprinkled with sessile golden glands; flowers disposed above the middle of the plant, borne on retrorse-hirtellous pedicels; lower calvx lip 2.5-3 mm. long at anthesis, 4-5 mm. long at maturity, the squama 3-3.5 mm. tall, concave, clothed like the stems; galea and tube 7-10 mm. long, the lower lip hirtellous in two lines, the lower filaments seated near the middle of the tube; nutlets brown or black, covered with peglike processes, banded.

LATERIFLORAE.—The following section is by no means as homogeneous as the species group termed Galericularia. However, the impression that the four species here associated with it are derivatives of S. lateriflora perhaps through hybridization cannot be avoided, despite the differences in conformation of the corolla. It will be noted that the corolla tubes of S. saxatilis and of S. arguta are enlarged somewhat and abruptly geniculate at the

ascendent pubescence; from S. lateriflora by the size of the flower. In Maine it occurs in Aroostook, Penobscot, and Androscoggin Counties. It has also been collected in New Brunswick (Carleton County) and in Quebec (Rimouski County).

7. S. lateriflora L. Sp. Pl. 598. 1753.—The range of S. lateriflora lies between 30° and 50° latitude, save in western Canada, where it ranges farther north. There are no records for Mexico. It is infrequent, however, south of 35°. It is most abundant in the drainages of the Ohio and St. Lawrence Rivers. A form occurs in which small capitate glands are sprinkled through the inflorescence and on the calvees in addition to the appressed hairs. This glandular form has been found as far south as Arkansas, Mississippi, and Georgia and as far west as Oklahoma and Minnesota. It would appear to center in Pennsylvania, being more frequently collected there than the eglandular form. It is equally abundant in New York, the New England States, and the maritime provinces of Canada. It is less frequent as one proceeds southward and westward. It is infrequent west of the Mississippi River and apparently does not occur west of the Plains States, save for one record in Arizona.

8. S. saxatilis Riddell, Cat. Ohio Pl. Suppl. 14. 1836, based upon specimens collected by Riddell on a dry hill 3 mi. southwest from Chillicothe, Ohio, and on arid cliffs in Kentucky opposite the mouth of the Scioto River; authentic specimens are in the Gray Herbarium, the National Herbarium and in the Torrey Herbarium.—S. Chamaedrys Shuttlw. ex Benth. in DC. Prodr. 12: 422. 1848 (a name published in synonomy).

A perennial herb spreading by threadlike stolons, its stems commonly 20-40 cm. long, weak and slender, usually reclining, spreading glandular in the lowermost parts, quite glabrous or sparingly hirtellous with appressed ascendent hairs in the

middle parts, the inflorescence, at least the pedicels, glandular with spreading hairs and often with ascending appressed ones, rarely quite glabrous; lowermost leaf-blades broadly ovate-cordate, tending to reniform, borne on petioles about as long as the blade, the median usually deltoid or deltoidovate; commonly 2-5 cm. long, crenate or serrate, borne on slender subequal petioles, passing rather abruptly into the diminished elliptical or ellipticallanceolate entire floral leaves, all usually glabrate, being ciliate on the margins, rarely quite glabrous. rarely thinly hirsute on both surfaces; flowers disposed in slender bracteate racemes 5-15 cm. long, some frequently on the axils of the upper leaves, borne on pedicels which are hirtellous with usually ascendent hairs and commonly with spreading glandular hairs as well; flowering calvees hirtellous and glandular, similar to the pedicels, the lower lip 2-2.5 mm. long at anthesis, 4.5-5 mm. long at maturity, the squama about 3.5 mm. tall, concave; galea and tube 14-19 mm. long, the lip glabrous, the tube sparingly pilose within along the under side; lower stamens seated 9-10 mm. above the base of the tube; nutlets a warm brown, covered with papillate processes.

A form with median leaves ovate, rather than deltoid, has been collected at Rockford and at Wilmington, Delaware. A form nearly glabrous, with diminished leaves and longer petioles has been collected near Nuttallburg, West Virginia. A similar form, quite eglandular, has been collected at Alderson, West Virginia. These are similar to Rügel's specimen from Warmsprings, Tennessee (S. Chamaedrys Shuttl.). Following is the known distribution of this species by counties: Pennsylvania: Fayette, Westmoreland, Beaver; Delaware: New Castle; Maryland: Montgomery; Virginia: Rockbridge, Pulaski, Arlington, Craig, Warren, Giles, Fairfax; West Virginia: Greenbriar, Fayette, Pocahontas, Boone, Jefferson; Ohio: Jefferson; Kentucky: Bell, Greenup, Monroe; Tennessee: Fayette, Polk, Blount, Cocke; South Carolina: "West Point."

9. S. arguta Buckley in Amer. Jour. Sci. 45: 175. 1843, based upon specimens collected by him on Black Mountain (Mt. Mitchell) in North Carolina.—S. saxatilis var. arguta Gray, Syn. Fl. N. Am. 2: 379. 1878, based upon the same.—S. saxatilis var. pilosior Benth. in DC. Prodr. 12: 423. 1848, based upon a specimen collected on Black Mountain by Rügel; the type is in the herbarium of the Royal Botanic Gardens at Kew.

A perennial herb spreading by threadlike stolons, its stems commonly 20-40 cm. tall, commonly branching near the base and ascendent, less often reclining, glandular throughout with rather dense spreading capitate glands and usually pubescent in the upper parts, even cinereous, with small retrorse eglandular hairs; lowermost leaf-blades broadly ovate-cordate, tending to reniform, borne on petioles about as long as the blade, the median ovate, somewhat cordate at the base, commonly 2-5

cm. long, sharply and strikingly serrate, borne on slender petioles as long as or even twice as long as the blade, passing rather abruptly into the diminished ovate and usually dentate floral leaves, all usually thinly hirsute on both surfaces; flowers disposed in slender bracteate racemes 5-15 cm. long, some frequently in the axils of the upper leaves, borne on pedicels which are clothed with retrorse glandular hairs; flowering calvees hirtellous with spreading hairs, both small eglandular ones and longer capitate glands, the lower lip usually 3-3.5 mm. long at anthesis, 5-5.5 mm. long at maturity, the squama about 3.5 mm. tall, concave, galea and tube 15-19 mm. long, the lip glabrous, the tube glabrous or sparingly pilose at the base above the angle; lower stamens seated 9-10 mm. above the base of the tube; nutlets a warm brown, covered with peg-like processes.

In so far as one may judge from the meager representations to be found in our herbaria, this species is relatively stable. It is doubtless highly localized. The flowers appear to be identical with those of S. saxatilis, save in the pubescence within the tube. However, it is difficult to perceive from dried specimens, even when boiled, what may be significant differences in conformation. As with S. saxatilis, the throat is seemingly open. The general habit is more strict than in that species. The nutlets are similar but not identical. I have seen only one example of a mature nutlet, together with three immature examples, and not many more than this of S. saxatilis. Judged on this slender basis, the patterns which the nutlets present appear to be significantly different, the processes of S. saxatilis being distinctly papillate and crowded, those of S. arguta more peglike and not crowded. The differences in pubescence and leaf-habit appear to be constant. The petioles appear to be constantly longer in proportion to the blade.

The known occurrence of S. arguta is as follows: Virginia: Montgomery, Blacksburg, Giles, Glenlyn; Tennessee: Unicoi, Unaka Springs; North Carolina: Yancey, Mt. Mitchell, Buncombe, Mon-

treat.

10. S. cardiophylla Engelm. and Gray in Bot. Jour. Nat. Hist. 5: 227. 1845, based upon specimens collected by Lindheimer in open woods near Houston, Texas, and by Drummond (no. 209); it is difficult to assign to a single specimen the rôle of "type." The Drummond plants in the Gray and Torrey herbaria are fragmentary and ill-pressed. Lindheimer gathered several specimens at different times, but Engelmann did not annotate those in his own herbarium. Perhaps the best choice of a standard would be Lindheimer no. 144 in the Gray Herbarium. It is very similar to a specimen in Engelmann's herbarium which bears the published data as to place of collection of the type.

An annual, 40-60 cm. tall, rather strict and stiffish, branching freely above the middle, the branches ascendent and strict, clothed with recurved, appressed hairs, particularly along the

angles, sometimes with spreading glandular hairs in the lowermost parts as well; basal leaves soon deciduous, the median deltoid, lightly cordate, commonly 2-5 cm. long, crenate-dentate, the lower borne on petioles as long as the blades, the uppermost subsessile and subentire, gradually diminished, the upper surfaces of all glabrous, the lower hispidulous with spreading hairs, their margins usually ciliate with longer hairs; flowers produced in the upper parts of the plant in the axils of gradually diminished leaves, the pedicels clothed like the stems; flowering calvees retrorse-pubescent and ciliate on the margins, the lower lip 4-4.5 mm. long at anthesis, 5-6 mm. long at maturity, the squama about 3.5 mm. tall, concave; galea and tube 13-22 mm. (generally about 18 mm.) long, the tube pilose

within, the lip glabrous; lower filaments seated 7-13 mm. (usually about 11 mm.) above the base of the tube; nutlets black, shallowly papillate.

Save for the variation in flower size which is indicated above, this species is fairly stable. It is possible that the plants of Arkansas represent a small flowered race with corollas 13-17 mm. long. The known range of this species by counties is as follows: Arkansas: Garland, Hempstead; Texas: Anderson, Galveston, Brazos, Montgomery, Smith, Cherokee, Harris, Matagorda, Colorado, Waller, Dallas, Walker, Robertson, San Augustine.

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# THE DUAL EFFECT OF AUXIN ON ROOT FORMATION 1

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Soon after the discovery that the root forming hormone as determined in the pea rooting test (Went, 1934) is identical with auxin (Thimann and Went, 1934, Thimann and Koepfli, 1935), it became clear that auxin is only one of the many specific internal factors involved in root formation. Several such factors have been listed and described elsewhere (Went and Thimann, 1937, Went, Bonner and Warner, 1938). In addition to the chemically well defined factors such as auxin, biotin, carotin, folliculin, vitamin B<sub>1</sub>, and amino acids, a number of experiments pointed towards the existence of a specific root forming hormone, rhizocaline (Went, 1936, 1938, Cooper, 1936, 1938). The concept of rhizocaline as used in this paper is essentially the same as it was when originated by Bouillenne and Went (1933) for "a special substance causing the initiation of roots," exclusive of chemically known substances such as auxin. The experiments suggested that the rhizocaline is redistributed inside the plant under the influence of auxin. The experiments of Cooper (1938) lead to the assumption that the root forming action of auxin must be two-fold. In the first place, the auxin causes an accumulation of rhizocaline near the point of application, and secondly, auxin activates or reacts with the rhizocaline. The present paper aims to present more and independent evidence for this dual effect of auxin on root formation.

EFFECTIVENESS OF DIFFERENT SUBSTANCES IN THE INDUCTION OF ROOT FORMATION.—In tables 1 and 2 the result of two experiments are recorded. For details concerning the technique of the experiments reference may be made to Went (1934). Stem cuttings of etiolated peas were treated either at their split tips or at their bases with a number of different substances, all known to be active in pro-

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ducing growth and all previously reported by Zimmerman and Wilcoxon (1935) to have root forming activity. That these substances should be retested on peas was desirable for two reasons: (1) quantitative data which might be directly compared with previous results were required, and (2) determinations carried out with auxin-free material were necessary. The experiments of Michener (1935) show that the latter condition is important. For ethylene seems effective in root formation only when auxin is present in the cutting. Therefore, in green herbaceous cuttings containing auxin, Crocker, Zimmerman, and Hitchcock (1933, 1935) found a very distinct rooting response to ethylene, while Michener found that in peas or Salix cuttings, poor in auxin, ethylene did not affect root formation.

From table 1 it appears that, of the various substances applied at the tip, indole acetic acid is by far the most active in causing root formation, after which, in order of decreasing activity, follow indole butyric and anthracene acetic acid. Indole propionic acid does not appreciably affect root formation. Since these substances become effective only at the base, their activity might be expected to depend on their transport rate inside the pea stem (Thimann, 1935). Recently the transport velocities for these substances in the Avena coleoptile were measured (Went and White, 1939), and the following values were obtained: indole acetic acid, 9.0 mm./hour; indole butyric acid, 6.5 mm./hour; anthracene acetic acid, 5.4 mm./hour; indole propionic acid, 4 mm./hour. Since the activities of these substances when applied at the base is approximately the same (only anthracene acetic acid is somewhat less active, according to table 2), it appears that the transport velocity is one of the main properties affecting root forming activity of a substance applied from the apex of a cutting. If the substances are applied at the base, differences in transport velocity do not seem to play an impor-

Table 1. Root formation (in roots per 10 plants) on etiolated pea stem cuttings. Treated for 15 hours at top or at base with various concentrations of various compounds. Each figure for 10 plants, except where noted.

	Applied at tip					Applied at base					
Compound	150 mg/l	30 mg/l	10 mg/l	2 mg/l	$_{ m H_2O}$	150 mg/l	30 mg/l	10 mg/l	2 mg/l	${ m H_2O}$	
Indole acetic acid		71.6 ± 7.6 Mean of 17		20		32	15.2 Mean of 60	9	3		
Indole propionic acid	14	8	5	9		10	7	1	6		
Indole butyric acid	39	29	10	20	$9.2 \pm 1.5$		17	10	4	8.2	
										Mean of 50 plants	
Indole valeric acid	••	3	2	11	Mean of 70 plants		10	7	3		
Anthracene acetic acid	38	6	1	7	ro piants	29	8	13	4		
Phenyl acetic acid	3	5	9	15		12	15	6	8		

tant part, and the different indole, anthracene, and naphthalene compounds all have a comparable ac-In another experiment, indole propionic acid was found to be much less active, just as in table 1, and in agreement with Thimann and Koepfli (1935). In the experiment of table 2, indole butyric appears to be slightly (although not mathematically significantly) more active than indole acetic acid, but this was not confirmed in other experiments. The decrease in activity at the highest concentrations of the indole compounds is due to toxicity, which was very marked with the indole valeric acid. Since in homologs permeability increases with increasing length of the C-chain (see e.g. Collander and Bärlund, 1933), the increased toxicity must be due to greater permeation.

This approximately equal activity of the indole, naphthalene, and anthracene compounds, when differences due to unequal transport are excluded, compares very well with the equal activity of these compounds in growth (Koepfli, Thimann and Went, 1938; D. Bonner, 1938) and seems to substantiate Thimann's rule (1935) that a substance active in the growth reaction will cause root formation as well. A notable exception, however, is phenyl

acetic acid. Since this exception constitutes a main point in the argument to follow, and also since it is of special importance in connection with the hypothesis that all substances causing growth, root formation, bud inhibition, etc., are doing so by taking part in one master reaction (Thimann, 1935), the effect of phenyl acetic acid on root formation has been determined in many different experiments, involving a total of many hundreds of pea stems. In no single experiment, however, has a significant increase in the number of roots formed under the influence of any concentration of phenyl acetic acid by itself ever been detected.

Analysis of the effect of indole acetic acid on root formation.—In pea stems the applied auxin tends to make the rhizocaline collect at the cut surfaces (Went, 1938). This should decrease its concentration in the middle portion of the stem. In lemon cuttings Cooper (1936, 1938) was able to demonstrate that when the auxin treatment was just right, the stem above the treated base could not root any more. This could be explained on the basis of a depletion of rhizocaline. Hellinga (1937) claims that Gouwentak's and his results (1935) do not support this view. However, they did not treat

Table 2. Root formation (in roots per 10 plants) on etiolated pea stem cuttings. Treated for 16½ hours at base with various concentrations of various compounds. Each figure is the mean of 10 plants, except where noted.

Compound	Concentration applied at base						
	1000 mg/l	300 mg/l	100 mg/l	30 mg/l	10 mg/l	3 mg/l	$ m H_2O$ Control
Indole acetic acid	110	149 ± 13	$35 \pm 6$	6	4	3	
		Mean of					
		45 plants					
Indole propionic acid	46	122	63	4	6		
Indole butyric acid	48	$161 \pm 26$	$61 \pm 10$	20 ± 5	1	5	
Indole valeric acid	0	28	14	6	********		$1.7 \pm 0.6$
							Mean of
							90 plants
Anthracene acetic acid	-	86	7	1	. 0		
Naphthalene acetic acid	-	83	5	3	5	-	
Phenyl acetic acid	1	1	3	2		-	

their cuttings with auxin previous to making the new cut surface. Also, the experiments of Pearse (1938) do not furnish evidence against the depletion hypothesis, since most of the developing roots grew from pre-existing root primordia.

In etiolated pea stems such a depletion was still more difficult to demonstrate. Only in the experiments which gave the best root formation after an initial indole acetic acid treatment was a decreased rooting response of the middle portion of the stem

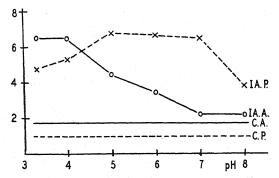


Fig. 1. Number of roots (ordinate) formed per pea cutting 10 days after treatment. Abscissa: pH of solution during 15 hour pre-treatment (IA.P.) or 15 hour after-treatment (IA.A.). The cuttings from IA.P. and C.P. were both after-treated with indole acetic acid 200 mg/l at pH 6; IA.P. were pre-treated with indole acetic acid 200 mg/l at various pH, C.P. with water at pH 6. IA.A. cuttings were after-treated with indole acetic acid 200 mg/l at different pH, C.A. with water at pH 6; the pre-treatment of both IA.A. and C.A. was with indole acetic acid 200 mg/l at pH 6.

noticeable. But whenever the root formation was less than 10 roots per plant (so that, according to Went (1938), enough rhizocaline was left to cause 40-10=30 roots to develop), no decrease in rooting response of the rest of the stem could be detected.

Table 3 shows an experiment in which pre-treatment for 15 hours with a high indole acetic acid concentration, followed by cutting off the basal 1 or 2 cm., decreased the root formation when the same stems were after-treated with indole acetic acid. Table 3 indicates also why in other experiments this effect was not observed. The pre-treatment with indole acetic acid was able by itself to induce root

formation in the basal zones when 1 or 2 cm. were cut off (after treatment with water). In many cases this rooting in the peas after-treated with water was so extensive that all other effects were overshadowed by it.

Whereas these experiments carried out under conditions of low rooting response of the peas cannot be used to demonstrate a depletion of rhizocaline in the upper parts of the cuttings, under just those conditions another phenomenon was discovered. It was observed in many experiments that the sum of the number of roots formed after either pre-treatment for 15 hours with auxin or 15 hours aftertreatment with auxin did not equal the number of roots formed after both pre- and after-treatment (30 hours) with auxin (see table 4). This means that the processes taking place during pre- and after-treatment are not identical. This same conclusion was reached for the pea test (Went, 1939), and there it was shown that the substances causing curvature not only took part in two reactions separated in time, but that also those reactions had a different pH sensitivity, the preparatory reaction being pH independent, the following growth reaction being the pH sensitive reaction. The pH dependence of the processes taking place during pre-treatment and after-treatment was therefore investigated for root formation also. Since there is no visible separation between these processes, a more or less arbitrary time interval was chosen for each of them. An experiment with a particularly clear result is presented in figure 1. The buffer solution somewhat decreased the rooting. It will be seen that when the pH of the indole acetic acid solution was varied during the pre-treatment (I.A.P.), the rooting response was somewhat less only at pH 3.3, 4, and 8, but around the pH of the cells (6) no effect of acidity was found. But for the after-treatment a pH dependence similar to that described by Bonner (1934) for the growth reaction existed. This can be explained along the same lines: during after-treatment only the undissociated molecules of indole acetic acid are active, and therefore the activity at pH 7 and 8 is very low.

THE EFFECT OF PHENYL ACETIC ACID AND CERTAIN NON-AUXINS ON ROOT FORMATION.—From the experiments of tables 1 and 2 it is evident that phenyl acetic acid by itself has no effect on root formation in pea cuttings. Considering (1) that in

Table 3. Root formation (in roots per 10 plants) on pea stem cuttings. Pre-treatment during 15 hours after which either 1 or 2 cm. of treated base was removed and the new base after-treated during 15 hours. Each figure mean of 20 plants.

After-treatment 15 hrs.	500 mg. indole ace acid per liter	tic Water
Pre-treatment 15 hrs.		of 1 cm. of 2 cm. of ut base cut base cut off off
500 mg. indole acetic acid per liter	69 55 151 138	31 30 6

Table 4. Number of roots formed (per 10 plants) on etiolated pea cuttings first treated basally with the solutions in the first column and afterwards (also basally) with the solutions in the upper row. Two experiments, the figures of the second in brackets. Each figure the mean of 10 plants, unless otherwise noted.

After-treatment 16 hours						
Pre-treatment 14 hours	200 mg. indole acetic acid/l		20 mg. indole acetic acid/l		Water	
Phenyl acetic acid 1000 mg/l	30 61	(44) (80)	11	(0)	0 1.4	(0) (11)
Water	0	(8)	0	(0)	Mean of 90 plants 0.8	(0)
					Mean of 90 plants	

green herbaceous cuttings it causes root formation (Zimmerman and Wilcoxon, 1935), (2) that a pretreatment with indole acetic acid increases the effectiveness of an after-treatment with the same substance, and (3) that in the pea test certain substances (hemi-auxins (Went, 1939)), not causing growth themselves, greatly increase the effectiveness of auxins, the effect of phenyl acetic acid during pre- and after-treatment was tested. Seven experiments, each involving hundreds of pea cuttings, gave qualitatively the same results, and two are reproduced in table 4 (see also table 5). It is evident from these results that phenyl acetic acid can take the place of indole acetic acid in the pretreatment, although quantitatively its effect is less. But in no case had it any effect as an after-treatment. This means that phenyl acetic acid can take part in only the first of the two successive reactions in which auxin takes part and which lead towards root formation. In this connection it is interesting to note that in two experiments there were indications that cutting off the base of a pea stem after pre-treatment with phenyl acetic acid, as compared to pre-treatment with water, decreased the subsequent response to indole acetic acid.

More striking still than the partial activity of phenyl acetic acid in root formation is the effect of certain substances which have no trace of growth promoting activity. Traub (1938) reported on the effectiveness of many substances, including sodium naphthol sulfonate, potassium anthraquinone sulfonate, anthranilic acid, and calcium furoate, in causing root formation on cuttings of Passiflora. Since the plants with which he worked were green and presumably full of auxin, experiments were performed in which these substances, kindly supplied by Dr. H. P. Traub, were tried on etiolated peas. Special tests showed that they were completely inactive in causing the growth reaction or the preparatory reaction in the pea test (see Went, 1939). But without doubt they all were active as pre-treatment for root formation if the peas were after-treated with indole acetic acid. By themselves, however, they did not give any root formation at all (see table 6). The same effect was found for  $\gamma$  phenyl butyric acid (table 5). Thus it has been demonstrated that there exist a group of substances which by themselves are not able to cause the formation of roots, but which greatly facilitate root formation in the presence of auxin or indole acetic acid. It also was found that this effect has to precede the actual root formation by auxin to be noticeable.

Discussion.—As mentioned in the introduction, earlier experiments by Went and Cooper demonstrated that the effect of auxin on root formation was not simple. Beyond participating in the differentiation of root primordia, at least one more reaction was recognized—namely, the redistribution or diversion of specific root forming factors (rhizocaline). In Went's experiments (1936, 1938) the accumulation of the rhizocaline at the place of auxin application or accumulation was demonstrated. Cooper's experiments (1936, 1938) showed how the remainder of the stem was emptied of rhizocaline after the auxin treatment.

Table 5. Root formation (in roots per 10 plants) on etiolated pea cuttings, pre- and after-treatment as noted and placed in 2 per cent sucrose.

After-treatment 22 hrs.	• 4				
Pre-treatment 20 hrs.		Indole ac			
Substance	Concer tration in mg/	* * * * * * * * * * * * * * * * * * * *	50 mg/l	Water	
Indole acetic acid	200	17	6	9	
Indole acetic acid	100	$12 \pm 1.8$	3 1	0	
Indole acetic acid	50	8	1	0	
Phenyl acetic acid	200	13	0	0	
Phenyl acetic acid		2	0	0	
Phenyl butyric acid	200	10	0	0	
Phenyl butyric acid		2	0	0	
Water		1	0	0	

In the present paper another method was used to demonstrate the dual effect of auxin on root formation. It was found by differentiating the effect of auxin in a first and second period of application that these effects were not additive, but that under the proper conditions neither pre- nor after-treatment had any visible effect by themselves. A combination of pre- and after-treatment was always more effective than the additive effects of either one alone. This difference in action could be made much clearer by using various substances during pre- and after-treatment. In the case of phenyl acetic acid it was clearly shown that, although this substance was unable to cause any root formation in the pea cuttings which were used, it was almost as

Table 6. Number of roots formed in 40 pea stems each. For the pre-treatment four concentrations (50, 100, 200 and 400 mg/l) of the three substances listed were used.

After-treatment for 24 hrs.	Indole acetic acid 100 mg/l	Water
Pre-treatment for 18 hrs.:		
Indole acetic acid	56	41
Sodium naphthol sulfonate	33	0
Potassium anthraquinone sulfonate.	37	0
Water	2	0

active during pre-treatment as indole acetic acid. No visible effect of this pre-treatment became evident unless the peas were after-treated with indole acetic acid or some other substance with true root-forming activity.

Since phenyl acetic acid has true growth activity, although lower than that of indole acetic acid (Koepfli, Thimann, and Went, 1938), it might be argued that its root forming activity was too low to be detectable in the tests. Such an argument is difficult to disprove, but with the highest concentration of phenyl acetic acid not even a trace of root forming activity was discovered. That it was effective as pre-treatment in much lower concentration is proven by tables 4 and 5. Other experiments, however, make it practically certain that in pre-treatment and after-treatment completely different reactions are involved. Many substances, like y phenyl butyric acid, sodium naphthol sulfonate, etc., which possess no trace of true growth promoting activity, are effective during pre-treatment (tables 5 and 6) but are absolutely ineffective during after-treat-

In all cases in which these substances, which are active during pre-treatment only, were found to cause root formation (Zimmerman and Wilcoxon, 1935; Traub, 1938), green cuttings containing auxin were used. From the experiments described above, the conclusion is evident that the auxin in those cuttings was sufficient to cause the after-treatment effect. Therefore, substances effective only in producing the first step towards root formation, but not actually taking part in the specific root

forming reaction, may seem active when the effectiveness of the auxin present in the cuttings has been increased. This is exactly what ethylene does (Michener, 1935). It therefore becomes evident that great care must be taken in drawing conclusions concerning root formation from experiments with green cuttings that contain auxin.

Evidence, although meager, has been presented to indicate that the pre-treatment reaction is identical with the redistribution effect on rhizocaline. The second reaction, which takes place during after-treatment, most likely consists of the reaction of auxin with rhizocaline, which results in the redifferentiation of the stem tissues into root primordia.

The very interesting investigations of Scott (1938) on the anatomy of differentiation of the tissues of the pea stem after application of indole acetic acid have shown that anatomically also the root formation goes by steps. First, a general meristem is formed, and in this meristem a further differentiation of root primordia takes place. It is not likely that the two successive reactions, described in this paper and leading towards root formation, can be identified with these two stages in anatomical differentiation. For one thing, the time relationships are very different. These observations, however, indicate that the physiological analysis can be carried much farther and that ultimately we may expect to find a long chain of reactions leading towards root formation, of which the two reactions which can be recognized are only two of the earlier links. This conclusion seems to be confirmed by the discovery of a great many factors involved in root formation (Went and Thimann, 1937).

A further comparison of growth and root forming reactions, and the bearing of these experiments on the problem of a master reaction induced by auxin, will be considered in another paper.

## SUMMARY

Etiolated pea stem cuttings were used as quantitative test objects for the root forming activity of different auxins. These peas are very suitable for such investigations, since they are relatively free of auxin. It was shown that phenyl acetic acid is not in itself a root forming substance.

If the period of auxin treatment is divided into two shorter ones, it is possible to separate two distinct portions of the effect of indole acetic acid in the formation of roots. The first phase of the auxin action is tentatively identified with redistribution of rhizocaline within the stem. This phase can be induced not only by indole acetic acid but also by phenyl acetic acid,  $\gamma$  phenyl butyric acid, sodium naphthol sulfonate, and other substances, not active in root formation proper. The second phase can be induced only by indole acetic acid and similar substances, and it has been suggested that this phase is the activation of the accumulated rhizocaline.

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# THE RELATION OF COPPER AND ZINC SALTS TO LEAF STRUCTURE <sup>1</sup>

# H. S. Reed

Tomato leaves show characteristic deficiency symptoms when the plants are grown from the seed-ling stage in nutrient solutions lacking traces of copper or zinc salts. These symptoms consist of specific types of curvature of the rachis or of the leaflets, dwarfness, and ultimate necrosis of tissue of the leaf. The alterations due to deficiencies of these elements are quite distinct and will be discussed independently.

The purpose of this paper is to discuss certain histological characters of affected tomato leaves in relation to problems of nutrition and growth. The plants utilized were kindly furnished by Mr. P. R. Stout and Dr. D. I. Arnon, who have conducted experiments in which extremely refined methods were employed.

The technic of the cultures was essentially the same as that described by Arnon (1938) and will not be repeated here, except to say that the complete list of microelements furnished was: zinc, copper, boron, manganese, molybdenum, vanadium,

<sup>1</sup> Received for publication October 11, 1938.

titanium, tungsten, chromium, nickel, and cobalt. Further details of technic are given by Stout and Arnon (1939).

Forbes' (1917) study of the effects of copper salts in irrigating waters and soils gives a comprehensive view of the physiological problem. He demonstrated that the copper salts were absorbed by the roots and traced the channels through which they traversed the plants. When lethal concentrations of copper salts were present the first injury was in the root-tip, where the concentration of copper was many times greater than the other parts of the plants. Forbes also showed that, when sublethal concentrations of copper salts were present, the growth of plants was enhanced.

The effects of copper salts on leaves was observed originally in connection with the results of spraying plants with copper-lime fungicides. Rumm (1893) compared the tissues in sprayed and unsprayed grape leaves, finding differences in width so small that they cannot be regarded as significant. The palisade parenchyma cells of the sprayed

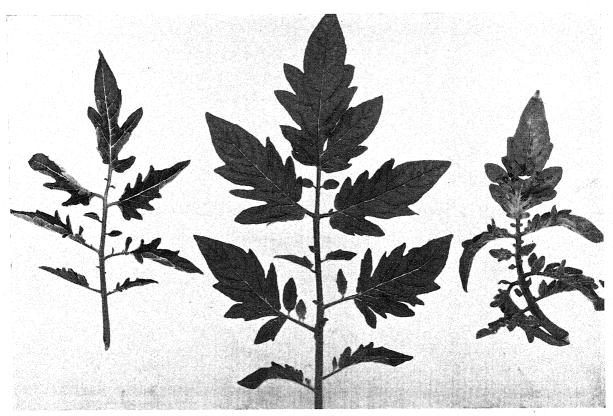


Fig. 1. Leaves of the same age from tomato plants grown in water cultures. From left to right the sources were: (a) complete nutrient solution with supplementary elements from which copper was excluded; (b) same with all supplementary elements present; (c) same with supplementary elements from which zinc was excluded. Photograph furnished by Dr. D. I. Arnon and Mr. P. R. Stout.

leaves, nevertheless, contained a greater number of chloroplasts, although their average size was somewhat smaller. The spongy parenchyma of sprayed leaves was more compact and also richer in chlorophyll.

Bain (1902) reported that the deposits of copper salts on leaves affected the formation of starch in the underlying tissues. Grape leaves showed the greatest benefit from copper sprays, apple leaves came next, and finally the peach, which is rarely benefited by spraying under normal conditions.

Bain made microscopical examinations of the leaves to determine the effect of the copper deposits on starch formation. When the leaves of seedling peach trees were sprayed with copper-containing liquids there was an irregular deposit of copper owing to the tendency to form droplets. Directly beneath these spots there was diminished starch formation both in the greenhouse and in the open. However, when sprayed leaves from trees in the open were examined, there was usually a halo of increased starch formation surrounding the spots especially of  $Cu(OH)_2$  and of  $Cu_3O_2(OH)_2$ .

It thus appears that with sufficient illumination the first effect of copper on the assimilative cells of the peach leaf, if not added in too great quantity, is to stimulate them to greater activity. Young leaves responded to the copper stimulus in less time than the older ones. There was a wide diffusion of the copper stimulus on the young leaves of peach and grape.

COPPER DEFICIENT LEAVES.—When one attempts to study the beneficial action of copper salts on the growth of seedling plants, it is necessary to use extremely refined methods. The salts and the water must be purified with especial care. Lipman and Mackinney (1931) showed that copper was essential for certain plants, but reported that the limit of impurity for each culture was less than 0.01 part per million of Cu.

Tomato plants began to show the effect of copper deficiency in a few weeks when grown under the refined technic employed by Stout and Arnon. The leaf shown at the left in figure 1 is from one of their cultures and shows how the axes and laminae were restricted in growth, although the number of leaflets and their lobes were not reduced. The midribs of the leaflets were not curved, but the laminae were characteristically involuted. The color of the leaflets at the time when the specimen was photographed was bluish-green, with a sheen very distinct from any other deficiency symptom yet discovered in the tomato.

In succeeding weeks necrotic areas appeared. The color of the young leaflets may be compared to the rich green color of citrus leaves in the early stages of exanthema.

The palisade cells of affected leaves were crowded with large plastids, confirming what was previously noted concerning the greenness of the leaf. The plastids contain starch grains and probably carry on normal photosynthesis. The plastids of young leaves were hyperchromatic, indicating that the first state of injury had already arrived. Moreover, these hyperchromatic plastids were surrounded by minute globular mitochondria.

Tomato leaflets bear stomata on both surfaces. Those on the dorsal surface are less numerous but larger than those on the ventral surface and are associated with cavities which are formed by the separation of the subjoined palisade cells. Relatively few of the dorsal stomata appear functional except during the early stages of their existence. The guard cells appear hypoplastic, and the majority of the stomata mentioned were filled by a mass of amorphous material.

The substomal cavities in the palisade parenchyma are initiated by a separation of the upper end of the palisade cells (fig. 2). In the same leaflet one may find advanced stages in the formation of these cavities in which the palisade cells are completely separated and the cavity still further widened by shrinkage in diameter of the cells adjoining the lacuna.

Tomato leaves from plants grown out of doors in a complete nutrient solution formed few stomata on the upper surfaces and there were no large substomatal cavities in the palisade parenchyma.

The formation of lacunae in leaflets of copperdeficient plants by the schizogenous process mentioned was followed by lysis of palisade cells, resulting in the formation of columniated parenchyma totally unlike that observed in plants from cultures lacking other essential elements (fig. 2).

The plastids of affected leaves were ultimately disorganized, evidence of which was shown by the development of pale stripes in the laminae along the veins, concomitant with an unthrifty condition of the plant. A microscopical examination of fresh sections of leaves showed that an extreme condition of hyperplasia existed. The palisade and spongy parenchyma cells did not generally accumulate neutral red in the vacuoles, but their nuclei were Spongy parenchyma cells had calcium oxalate crystals of the monohydrate type. plastids of all cells were in various stages of disorganization with a tendency to form clumps at one end of the cell. Most of them had lost their homogeneity due to the aggregation of the chlorophyll into small droplets which gave the plastids verrucose surfaces. The green pigment in the droplets was readily dissolved out by dilute sodium oleate or by dilute commercial liquid soap. The cell vacuoles after such treatments were completely filled with diffuse pale green pigment.

Tomato leaves in a more advanced stage of injury showed necrotic tissue. An examination of sections

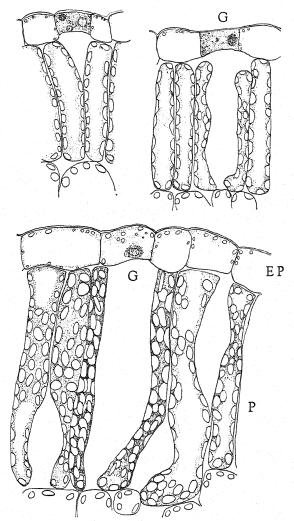


Fig. 2. Palisade parenchyma of tomato leaves to show successive stages in the formation of lacunae in copper-deficient plants. Drawings from fixed material stained with acid fuchsin and methyl green. The upper figures represent conditions in younger leaflets. The lower figure represents a condition characteristic of older leaflets. EP, epidermis; G, guard cell; P, palisade.

of fresh material showed that disorganization and collapse of cells started in the palisade parenchyma, usually in the vicinity of stomata, thence spreading to the spongy parenchyma where similar disintegration occurred, and finally the cells turned brown and dried out.

The copper content of orange leaves indicates that there may be some relation to exanthema, although soil and climatic conditions are also important factors (Haas and Quayle, 1935). Leaves from healthy trees contained about 15 parts per million copper, and leaves from affected trees in the same locality contained 7 parts per million.

Exanthema is often cured by the application of copper sulfate to the soil or to the trees, but the way in which it acts is not well understood. The

low copper content of affected pear leaves suggests that exanthema was due to a deficiency of copper (Oserkowsky and Thomas, 1933), yet healthy appearing leaves selected from trees which showed signs of the disease were no richer in copper than the affected leaves. However, normal leaves from localities free from the disease contained from two to four times as much copper as the typically exanthema leaves.

In early stages of exanthema the leaves are often dark green and appear healthy, but they are usually followed by leaves and shoots which are depauperate and short-lived. The leaves of affected orange trees are spotted with exuded gummy or resinous material. Fruits on affected trees fail to develop.

One cannot fail to be impressed by the importance of the function of copper in plant nutrition, although comparatively few physiological or cytological studies have been made. The principal attention thus far paid by plant biologists has been to the toxic effects of copper. It is evident that future investigations should have a broader scope than those of the last few decades.

ZINC DEFICIENT LEAVES.—Additional studies on the cytology of zinc-deficient tomato leaves were made on material furnished by Mr. Stout and Dr. Arnon. The leaf at the right in figure 1 shows acute symptoms of zinc deficiency, among the most prominent of which are: (a) dwarfness, (b) paleness, (c) downward curvatures of the leaflets, (d) involuted laminae, (e) dead spots on rachis and laminae.

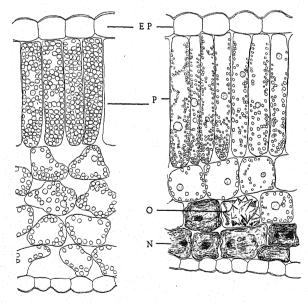


Fig. 3. Structure of tomato leaves drawn from fresh sections mounted in Ringer's solution. Left, a healthy leaf from a plant grown in nutrient solution containing all supplementary elements; right, a pale, dwarfed leaf from a plant grown in nutrient solution with supplementary elements from which zinc was excluded. EP, epidermis; N, necrotic cells; O, mass of calcium oxalate crystals; P, palisade.

These characteristic symptoms were produced also on tomato plants grown in a soil in which the little-leaf of fruit trees is found. It is important therefore to compare the histological structure of plants from Delhi soil with these which grew in nutrient solutions deficient in zinc.

The palisade parenchyma of affected tomato leaves grown in Delhi soil was greatly hypertrophied as a result of the striking elongation of the cells and contained relatively few plassids (Reed, 1938). The spongy parenchyma, on the contrary, was poorly developed and comparatively chlorotic. These histological characters have been noted in citrus and other leaves affected with little leaf symptoms (Reed and Dufrenoy, 1933).

The structure of healthy and affected tomato leaves from plants grown in nutrient solutions, therefore, have a general resemblance to those from soil, though the contrast is less pronounced. Figure 3 shows the structure of healthy and affected tomato leaves from plants grown in the nutrient solutions of purified salts previously mentioned. The drawings were made from fresh sections mounted in Ringer's solution, and the same scale of magnification was used throughout.

The palisade cells of the affected leaves were longer and narrower and the spongy parenchyma was more compact than that of the healthy leaf. Not more than one palisade cell in seven had the ability to accumulate neutral red in its vacuole, indicating a hypoplastic condition. The plastids contained fat globules, but there were no free globules in the cell vacuoles. The nuclei stained readily with methylene blue, even though the cell was notably disrupted.

The number and size of the plastids showed a contrast. Those of the palisade of the affected leaf were small and had a tendency to accumulate at the lower end of the cell. Those of the healthy leaf were larger, contained starch grains instead of fat globules, and were uniformly distributed in the parietal cytoplasm. In affected leaves where the processes of disintegration had been operative, the number as well as the size of the plastids was greatly reduced, producing pale or mottled leaves which are characteristic of zinc deficiency conditions.

It is important to notice how intimately the production of chloroplasts is related to the presence of zinc salts in practically every plant thus far examined. Iron salts are also important, but in none of the cases examined was their presence a limiting factor for chlorophyll production. Mottled orange leaves (to cite one example) contain as much iron as healthy leaves. The addition of iron does not ameliorate the condition.

The most conspicuous indications of degeneration in the tomato leaf were in the spongy parenchyma, where more of the cells contained aggregations of calcium oxalate crystals than those of healthy leaves. In this layer the first signs of necrosis were found, consisting of shrinkage and formation

of a melanotic substance which obscured the details of the cell structure (fig. 3). The necrosis advanced from the spongy parenchyma, ultimately reaching the dorsal surface and producing the brown spots previously mentioned. Toluylene blue penetrated readily and stained the degenerated cells of the spongy parenchyma but did not penetrate the palisade cells until they were dead. In affected orange leaves the formation of calcium oxalate was a forerunner of phloem necrosis, and it may indicate an incomplete utilization of carbohydrates (Reed and Dufrenoy, 1935).

The degeneration of tomato leaves showing zinc deficiency starts in the spongy parenchyma in contrast to the degeneration of the palisade layer in copper deficient leaves described above.

Plants in purified nutrient solutions showed necrosis of the rachis of the leaves after a time. In the first well defined stages the necrosis was restricted to the chlorophyll-bearing cells situated inside the epidermis. In later stages the deeper layers of the rachis were necrotic. The affected cells contained tannin (reaction with ferric chloride or potassium bichromate). The epidermal cells showed no injuries until after the hypodermal layers had collapsed. The hypodermal and collenchyma cells had no capacity for storage of neutral red. Collapse of the hypodermal cells was due

to weakening of the radial walls and destruction of cellulose.

### SUMMARY

Tomato plants grown in copper-deficient nutrient solutions showed characteristic dwarfing, involution of the leaflets, color change, and eventual necrosis. At an early stage substomal cavities were formed, resulting from the separation of palisade cells. Subsequently the separated palisade cells shrunk and ultimately disappeared as a result of the lysis of the contents, producing columniated palisade tissue. If necrosis appeared, it started in these schizogenous cavities.

Tomato plants grown in zinc-deficient nutrient solutions showed characteristic dwarfing, curvature of leaflets, chlorotic leaflets, and involuted laminae in which severe necrosis appeared early. The palisade cells were longer and the spongy parenchyma was more compact than in similar leaves from healthy plants to which zinc had been supplied. The plastids were small and contained oil drops, but there were no free oil globules in the vacuoles. Conspicuous signs of disrupted metabolism were seen in the spongy parenchyma, identifiable as scarcity of plastids, production of melanotic material, and abundance of calcium oxalate crystals.

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# CULTURE STUDIES OF MARINE ALGAE. I. EISENIA ARBOREA <sup>1</sup>

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EISENIA IS a typical member of the Laminariales. As stated by Setchell and Gardner (1925), there are two well recognized species in the genus, E. arborea Areschoug, the type species, found along the coast of southern California, and E. bicyclis (Kjellm.) Setchell, found in Japanese water. Setchell and Gardner do not mention E. Cokeri, described by Howe (1914) from the coast of Peru, where it is reported as very abundant. I have not examined specimens of E. Cokeri. Howe states that the plant is very similar to E. arborea, but that the stipe is longer in the Peruvian plant, and that the sporophylls are "longitudinally rather than reticulately rugose." My observations indicate that the stipes of typical plants of E. arborea are fully as long as measurements given by Howe for E. Cokeri. Furthermore, the sporophylls of E. arborea are decidedly longitudinally rugose or corrugated in most cases. In fact, Howe's figures (Plate 14A, 15, 16) represent a plant essentially identical with those found along the coast of southern California. Howe states that the most distinctive feature of E. Cokeri is the almost complete lack of muciferous canals in the stipe. These structures are usually very abundant in E. arborea, forming a macroscopically visible ring near the outer margin of the stipe as seen in cross section. This is the only difference which I can discover between Eisenia Cokeri as figured and described by Howe and the southern California plant. It seems doubtful, therefore, if E. Cokeri should be considered as a distinct species.

Eisenia (?) Masonii described by Setchell and Gardner from the Revillagigedo Islands off the west coast of Mexico is a doubtful member of the genus.

Eisenia arborea was first described by Areschoug in 1876. It is very common at low tide level on rocky shores along the coast of southern California, mostly south of Redondo Beach. The sporophyte is well described by Setchell and Gardner (1925). It is commonly 12-14 decimeters long, and is frequently 15 decimeters or more in length. In one case the stipe alone measured 15 decimeters. Clare and Herbst (1938, p. 494) state that the plant is "usually one-half to three-fourths of a meter in They may have had reference to measurements given by Setchell and Gardner (1925), who state that the stipe is commonly 1 meter long. At any rate, the statement of Clare and Herbst is very misleading, especially when accompanied by a photograph of a plant with an unusually short stipe.

Setchell (1905) has described the post-embryonal development of the sporophyte of *Eisenia arborea* in which it passes successively through stages in which it assumes the aspect of a *Laminaria* and later of an *Ecklonia* with lateral pinnae before it develops the characteristic features of *Eisenia*.

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Post-embryonal stages of *Eisenia bicyclis* as described and figured by Okamura (1927, p. 140-143, pl. 238) are very similar to those of *E. arborea*.

Numerous collections of fruiting sporophytes have been made at all seasons of the year during the past four years. The normal fruiting period seems to be from about May to December. Vigorous cultures have been started from plants collected as early as June and as late as December. From January to May only occasional remnants of old sori are usually to be found.

The sporophylls are linear and mostly unbranched, but forked sporophylls are frequently encountered. The sporophylls are narrowed at the base and rounded at the apex. The margin is usually beset with a series of low teeth, but is frequently nearly smooth. Marginal proliferations are more rarely present. Any or all of the leaflets may function as sporophylls when mature. The sori are elongate patches, typically covering both sides of the basal third or more of the sporophyll, except for a narrow sterile strip along the margin. In old sporophylls the sorus may be limited to an area some distance from the base. The latter condition apparently results from the activity of a meristematic region at the base of the sporophyll and the erosion of the tip. The sori are frequently irregular in outline or even composed of discontinuous patches, but I am unable to agree with the statement of Clare and Herbst (1938) that the sori first appear as irregular patches which later fuse. My observations indicate that the sori are typically entire and continuous from the beginning. The maturity of the sori may be roughly judged by the depth of color. Fully mature sori are dark in color and more mucilaginous when handled, especially some time after removal from the water.

Ikari (1926) made culture studies of Eisenia bicyclis. He showed that the life cycle is in general much like that previously described for other members of the order. He does not give many details concerning the gametophyte generation. However his findings are in close agreement with those here reported for E. arborea.

Culture studies of Eisenia arborea have been in progress for the past four years. As this paper neared completion there appeared an article in this Journal by Clare and Herbst (1938) which deals with culture studies of Eisenia arborea. These investigators fail to mention the work of Ikari. Their observations were based entirely on living material. The study here reported includes a cytological investigation of critical stages in the life cycle. This report differs, furthermore, in a number of important respects from the findings of Clare and Herbst and includes items either overlooked or unobserved by them.

CULTURE METHODS.—Large Petri dishes and glass stacking dishes 4 cm. high and 12 cm. in

diameter were employed with nearly equal success. There is reason to believe that the cultures receive a better supply of oxygen in shallow containers. Culture solutions were composed of sterile sea water to which was added small amounts of NaNO3 and Na<sub>2</sub>HPO<sub>4</sub> as recommended by Schreiber (1930) and other investigators. Most investigators have reported temperatures of 10-12°C. as necessary or most favorable for the culture of germling stages of Phaeophyceae. Kanda (1938) and a few other investigators have cultured germlings of Laminariales at ordinary room temperatures. In the case of Eisenia arborea an abundance of fruiting gametophytes and young sporophytes developed in cultures at ordinary room temperatures (mostly 17-22°C.). Cultures kept at lower temperatures developed more slowly and less vigorously. They did not fruit as readily nor as vigorously. In view of the fact that I have also been able to culture germlings of several other brown algae at room temperatures, it seems possible that the importance of low temperatures may have been over-emphasized in some instances by previous investigators. However, most investigators of germling stages in the Laminariales have dealt with plants adapted to relatively low temperatures. In southern California the surface temperature of the ocean along the shore varies mostly around 14-17°C. It seems likely that the temperature to which Eisenia arborea is accustomed in nature may account for the readiness with which the sexual plants developed at relatively high temperatures in the cultures. The cultures were kept in a well lighted room with northern light exposure.

Mature fruiting sporophylls were kept out of water from 1 to several hours with some precautions taken to prevent excessive drying. Three or 4 small pieces were quickly but thoroughly washed in sterile sea water and then placed in a small amount of nutrient solution for 2-3 minutes. In the case of many brown algae, especially members of the Laminariales, this is sufficient time for the discharge of mature spores if fully mature fruiting portions have been selected. The pieces were then removed and a few cubic centimeters of the nutrient solution, containing great numbers of motile spores, were transferred, by means of a pipette, to other dishes containing about 100 cc. of nutrient solution and several glass slides.

Clare and Herbst (1938) and a number of other investigators have followed a practice of leaving bits of fruiting sporophylls in the nutrient solution in the culture dishes for 12-24 hours. This is entirely unnecessary and undesirable in the case of many brown algae, and it is certainly so in the case of Eisenia. This procedure apparently accounts for some of the difficulties these investigators have experienced, especially in studying motility of the zoöspores and in keeping the cultures free from contamination. Clare and Herbst report placing "10 to 12 fragments of sori about 1 centimeter square" in each culture dish. My experience indi-

cates that if fully mature sori are used, this procedure would most certainly result in overcrowding of the cultures. It is well known, moreover, that copious quantities of mucilaginous material are liberated from even small pieces of many different members of the Phaeophyceae when such pieces are left standing for even a short period of time in water. This mucilaginous material undoubtedly encourages the development of bacteria and perhaps other undesirable inhabitants of the cultures. Even with the best of precautions, however, it is very difficult to get cultures altogether free from diatoms. Chytrids also frequently infect the cultures. I have several times obtained vigorous cultures of germlings in which I was unable to detect a single diatom, but these cultures never fruited. It is doubtful if their failure to fruit is of any special significance since other cultures with diatoms present also failed to bear sex organs. It is of interest to note in this connection, however, that Angst (1927) observed that those cultures of Costaria containing infusoria made the most rapid progress.

Development of sporangia.—Small portions of sori from freshly collected plants and in various stages of development were fixed in a solution composed of 1 gm. of chromic acid and 1 cc. of glacial acetic acid per 100 cc. of sea water. N-butyl alcohol was employed in dehydrating and embedding, and sections  $4\mu$  thick were made in the usual manner and stained with Heidenhain's iron-alum-haematoxylin.

In sterile portions the cortex is composed of 1 or 2 rows of small cells, the cells of the outer layer being somewhat elongated perpendicular to the surface of the blade. In the development of fruiting areas the cells of the outer layer divide, usually slightly obliquely, forming an outer cell, which is still somewhat elongated vertically, and a smaller, more or less isodiametric inner cell. The outer cell contains 5 or more chromatophores and a single nucleus. It is destined to become a paraphysis. Its outer wall very early begins to thicken (fig. 2) to form the so-called hyaline appendage typical of the paraphyses of the Laminariales. The inner cells each contain a single nucleus and 2-3 or occasionally 4 chromatophores. These cells each give rise to a sporangium. The nucleus enlarges considerably and divides (fig. 1). During this division about 30 minute chromosomes are visible. One daughter nucleus migrates into a small protrusion which has meanwhile developed outwardly between adjacent young paraphyses (fig. 1) and into which a single chromatophore has previously migrated. A cross wall develops, and the chromatophore of the resulting sporangial cell divides to form several chromatophores. At the same time, the nucleus enlarges greatly and undergoes typical meiotic stages (fig. 3-5). Synaptic union reveals the presence of 15 pairs of minute chromosomes (fig. 5). In early meiotic stages the chromosomes are elongate and present the appearance of a typical spireme. Each apparently shortens during synapsis to form a spherical or slightly elongate body. The two nu-

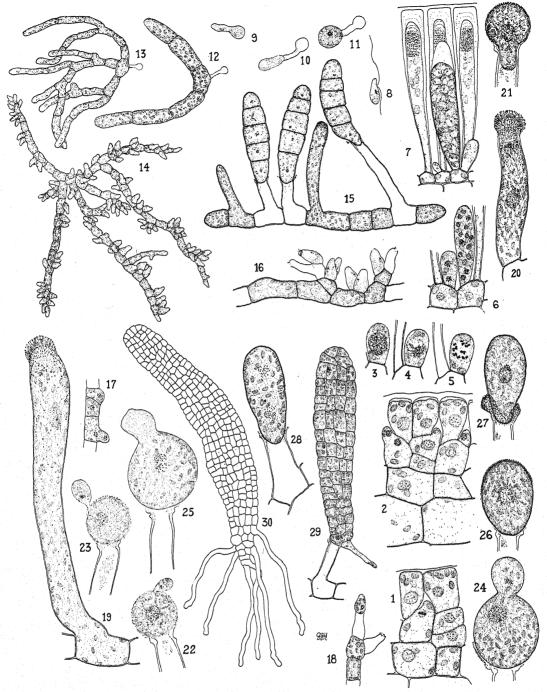


Fig. 1-30. All figures were drawn with the aid of an Abbé camera lucida.—Fig. 1-2. Section of young sorus of Eisemia arborea perpendicular to the surface, showing early stages in the formation of sporangia and paraphyses. ×700.—Fig. 3. Spireme stage during meiosis in a young sporangium. ×700.—Fig. 4. Synizesis. ×700.—Fig. 5. Young sporangium showing chromosome pairs at diakinesis. ×700.—Fig. 6. Later stages in the development of sporangia. ×700.—Fig. 7. Section of mature sorus perpendicular to the surface, showing mature sporangium, young sporangia, and paraphyses. ×700.—Fig. 8. Zoöspore. ×700.—Fig. 9, 10. Germinating spores 12 hours after release from the sporangium. ×700.—Fig. 11. Germling 10 days old. ×700.—Fig. 12. Female gametophyte of 23 days. ×325.—Fig. 13. Male gametophyte of 23 days. ×325.—Fig. 14. Male gametophyte 50 days old with numerous antheridia. ×325.—Fig. 15. Female gametophyte 50 days old with young sporophytes and developing oögonia. ×325.—Fig. 16. Portion of male filament 50 days old with mature and empty antheridia. ×700.—Fig. 17. Early stage in the formation of antheridia. ×700.—Fig. 18. Empty antheridia with extruded antherozoid still in contact with the tip of an antheridium. ×700.—Fig. 19, 20. Mature oögonia showing papillate tip. ×700.—Fig. 21. Egg emerging from the

clei resulting from the first division of this primary nucleus contain the haploid number of chromosomes (fig. 6). I have also been able to distinguish about 15 chromosomes in the nuclei of a fournucleate sporangium. Following the first nuclear division, all subsequent nuclear divisions in a given sporangium are simultaneous. By the time 16 nuclei have been formed it becomes apparent that the number and position of the chromatophores correspond closely to that of the nuclei, each chromatophore being closely associated with a single nucleus (fig. 6) and dividing with it, as reported by McKay (1933) for Pterygophora. As the sporangia approach maturity, numerous small vacuoles about 0.5 \( \mu \) in diameter appear in the cytoplasm between the nuclei. The individual zoospores are soon delimited, apparently by fusion of these vacuoles. In mature sori the paraphyses are unicellular and contain several chromatophores and a single nucleus. The tip of the cell is often filled with a dense protoplasmic mass below which are the chromatophores. As the sorus reaches maturity, the cuticle covering the tip of the paraphyses loosens and is shed. Mature sporangia measure 45-60  $\times$  12-15  $\mu$  and appear to contain approximately 32 or 64 spores. I find no evidence that the contents of mature sporangia draw away from the wall as reported by Clare and Herbst. The wall at the tip swells greatly (fig. 7) as the sporangium reaches maturity, and eventually ruptures to release the spores. New sporangia are continually arising at the base of old ones from the same supporting cells, and it seems probable that a given sorus continues to shed spores for some weeks at least.

Zoöspores.—Clare and Herbst (1938) report that they were unable to detect flagella on the zoöspores or any evidence of motility. They state that the spores "emerge and float away." They furthermore state that the spores float for 12-24 hours before becoming attached. These statements and conclusions seem to be very much in error. Their inability to detect motility of the zoöspores or to see the flagella would appear to be due to faulty technique in starting cultures, or as I have already suggested, to the use of immature sori, or perhaps to both. I have obtained vigorous swarms of very active spores some 20 or more different times. There is, moreover, not the least difficulty in seeing the two flagella. These are readily visible without resort to staining. In fact, some staining procedures will quickly cause the zoöspores to shed their flagella. In this connection one is also inclined to question the findings of Herbst and Johnstone (1937), who report their inability to find flagella

or evidence of motility of zoöspores in the case of Pelagophycus porra (Leman) Setchell.

The zoöspores of Eisenia arborea (fig. 8) are of the usual form, slenderly pyriform, 6-9 μ long and 3-4.5 \mu in diameter, with two lateral flagella inserted somewhat nearer the anterior end. The forwardly directed flagellum is, as usual, the longer and more active. It is at least twice the length of the cell. The posteriorly directed flagellum is only slightly longer than the cell and is practically motionless while the swarmer is motile, but it may at times exhibit movement. A single chromatophore occupies the posterior third or more of the cell; the anterior end is hvaline. A stigma is definitely present in the form of a more or less elongated body associated with the chromatophore on one side of the cell. The figures of Ikari (1926) show that the zoöspores of Eisenia bicyclis are very similar to those of E. arborea. According to a translation of his paper. Ikari reports the absence of a stigma in the motile spores of E. bicyclis, and none is included in his figure of the spores. Kanda (1936, 1938) reports the absence of a stigma in the zoospores of a number of Laminariales which he studied. He furthermore states that the stigma has been previously reported as present in the zoöspores of only three members of the order: namely in Laminaria religiosa, reported by Ikari (1921); in Egregia Menziesii, reported by Myers (1928); and in Pterygophora californica, reported by McKay (1933). In addition, Kanda (1938) reports having observed a stigma in the zoospores of Chorda filum (L.) Lam'x. In spite of the presence of a stigma in the zoöspores of Eisenia arborea I have been unable to detect any special phototactic response on the part of the swarmers.

GAMETOPHYTES.—Within a few hours, and usually within an hour or so after liberation, the swarmers come to rest, lose their flagella, and become round in outline. Secretion of a wall soon attaches them to the substrate. Germination of the spore is very much like that previously described for other members of the Laminariales. Under favorable conditions a germ tube (fig. 9) is formed within 8-12 hours, but its formation may be delayed as much as 20-30 hours. The tip of the tube swells, and most of the protoplasm migrates into the swollen tip (fig. 10) and is cut off from the original spore case by a cross wall in a manner characteristic of the order. This occurs when the germlings are 36 hours to a few days old. There follows a period of increase in the size of the cell (fig. 11) and in the number of chromatophores—a developmental phase which Harries (1932) and other investigators speak of as a temporary resting period. A short filament

tip of an oögonium, showing the gelatinized tip of the oögonium stretched over it as a thin veil. ×700.—Fig. 22-25. Stages in fertilization. ×700.—Fig. 26. Oöspore, showing a remnant of the gelatinized tip of the oögonium forming a cap over the distal portion. The zygote nucleus shows the diploid chromosome number. ×700.—Fig. 27. Oöspore showing the ruptured apex of the oögonium surrounding its base. ×700.—Fig. 28. Young sporophyte, showing nuclei with diploid chromosome number. ×700.—Fig. 29. Embryo sporophyte of a single layer of cells forming a first rhizoid. ×325.—Fig. 30. Embryo sporophyte nearly one-half millimeter long and still monostromatic. ×162.

is soon formed by cell division. It usually grows laterally in opposite directions from the original proembryo (fig. 12). Although the germ tubes do not exhibit any noticeable phototropic response, the first formed filaments of the gametophytes show a distinct positive phototropic growth in moderate light intensities. This usually results in a unilateral branching of the gametophytes (fig. 13, 15). Under favorable conditions, male and female plants are distinguishable about 15 days after germination. The male plants are conspicuously smaller and, as in other members of the order, considerably more branched than the female plants. An entire culture will frequently develop into very elongate, branched filaments with few or no sex organs. This condition is interpreted as one favorable for growth but unfavorable for reproduction. Harries (1932), working with Laminaria digitata, reports that nutrient conditions most favorable for growth are also most favorable for the production of sex organs. She indicates, however, that high temperature, intense light, or an excessive amount of phosphates induces development of an elongate type of gametophyte. Schreiber (1930) reports that cultures of germlings of Laminaria, which were sterile at higher temperatures, were induced to fruit by placing them in a refrigerator at 2-4°C. When gametophytes of Eisenia arborea in the cultures once take on an elongate sterile form, I have been unable to induce the formation of sex organs, although I have tried low temperatures (2-3°C.) and changes in light intensity as well as changes in nutrient concentra-

Fruiting female fllaments measure  $11-14~\mu$  in diameter. This is somewhat larger than measurements given in the case of gametophytes of most other members of the order, but corresponds closely to measurements given by Ikari (1926) for gametophytes of Eisenia bicyclis. Clare and Herbst (1938) give no measurements of mature gametophytes. Female gametophytes of Eisenia arborea are frequently unbranched and composed of relatively few cells, but they are also frequently composed of numerous cells and bear one to several branches. The cells are mostly 1.5-3 diameters long. Male filaments are usually  $6-8~\mu$  in diameter with cells of a corresponding length.

Development of antheridia.—Antheridia in the Laminariales were first observed by Sauvageau (1915), who described them as developing in clusters at the tips of the branches of the male filaments. This characterization has been accepted ever since with but little modification in most descriptions of antheridia in the order. In some cases it seems evident that antheridia were either not seen or were not carefully studied. The photomicrograph of so-called antheridia published by Clare and Herbst (1938, fig. 3) for Eisenia arborea is certainly open to question. One is inclined to wonder if these investigators may not have observed male filaments with terminal branches infected with chytrids, since they state, as have some other investigators, that

any cell may function as an antheridium and that the filaments become "invisible" soon after discharge of the gametes.

I have studied antheridial development of Eisenia arborea, both in living cultures and in stained slides. In vigorous cultures they first appear about 40 days after germination of the spores. The nucleus of a cell of a filament divides and one of the daughter nuclei migrates into a short protrusion of the cell. It is then cut off by a cell wall which divides the cell obliquely (fig. 16, 17) or separates the protrusion at the base from the parent cell. In either case the daughter cell receives a number of chromatophores. It may function as an antheridium or it may be the initial cell of a very short branchlet which produces one or more antheridial outgrowths at its apex. As a result, the more or less conical antheridia are sessile either on the branches or on very short 1-2-celled branchlets. They arise in clusters of 2-3 or more on a given cell. New ones may form after older ones on the same branchlet have discharged their antherozoids. In vigorous cultures the antheridia are not at all limited to the tips of the branches as reported by Clare and Herbst (1938) but may extend the entire length of the branches (fig. 14). Harries (1932) states that in Laminaria digitata "any cell may function as an antheridium, the end cells being first converted." Herbst and Johnstone (1937) and Clare and Herbst (1938) use nearly the same words in describing the antheridia of Pelagophycus porra and Eisenia arborea, respectively. These statements give the impression that the antheridia consist of the transformed terminal cells of the filaments, resembling in origin a uniscriate plurilocular sporangium. This is contrary to the descriptions and figures given by most investigators, and it is certainly not the case in Eisenia arborea. My observations indicate that the cells of the branches giving rise to antheridia may lose their contents following the discharge of the antherozoids, but no evidence was obtained which would indicate that the cells of the branches function as antheridia or are converted into antheridia. In the case of very short lateral branchlets a cell of the branchlet is occasionally found to be continuous with an empty antheridium (fig. 16), suggesting that a cross wall failed to form at the base of the antheridial outgrowth.

As the antheridium nears maturity, the wall at the tip becomes greatly thickened. When fully mature, this thickening consists of a nearly spherical globule of gelatinized wall material (fig. 16). This spherical cap evidently becomes loosened as the result of a transverse rupture encircling the base of the plug or cap, and the antherozoid is released, leaving the tip of the antheridium open.

ANTHEROZOIDS.—All evidence indicates that in the Laminariales each antheridium produces but a single antherozoid, although the liberation of the antherozoid has been seen by only a few investigators. McKay (1933) studying Pterygophora, gives

the first good description of male gametes of the Laminariales, although Williams (1921) had previously observed the liberation of these motile cells in Laminaria. I have not been fortunate enough to see the antherozoids discharged from the antheridia and hence cannot be certain that a number of small motile cells with two lateral flagella of nearly equal length and a number of very pale chromatophores, which were seen in the cultures, were male gametes of Eisenia. It is believed that they were. However, in stained slides of fertile gametophytes I have been able to find cases of single antherozoids killed just after extrusion, while still in contact with the tip of the antheridium (fig. 18). Furthermore, in living plants I have observed, in each of a number of undischarged antheridia, a single minute reddish stigma, and in stained slides antheridia always contain but a single nucleus. These observations indicate rather definitely that but a single antherozoid develops in each antheridium. It is of interest to note that Kanda (1936, 1938) reports the absence of a stigma in the antherozoids of several members of the Laminariales which he studied.

Sauvageau (1916) thought he observed two small pale chromatophores in the antheridium of a species of Laminaria. Most investigators have either concluded that there is but a single chromatophore or are silent on this point. Kanda (1938) observed antheridia and antherozoids of a number of Japanese members of the Laminariales. He states that some of the apical cells of the male filaments become antheridia and that most of the chromatophores disintegrate as the antherozoid is formed. He thus indicates that there are several chromatophores in the antheridium when first formed. As I have previously indicated, the antheridia of Eisenia arborea are not to be considered as transformed terminal cells of the filaments. Nevertheless, the antheridia seem to contain a number of chromatophores at the time of their formation, although these are pale and indistinct. In mature undischarged antheridia and in discharged antherozoids stained slides likewise reveal the presence of a number of chromatophores (fig. 18). Finally, during stages in the fusion of the antherozoid with the egg (fig. 22-25), a number of chromatophores are distinctly visible in the antherozoid. It seems evident, therefore, that in the case of Eisenia arborea the chromatophores in the antheridia do not disintegrate as reported by Kanda for certain members of the order.

Formation of oögonia.—Female gametophytes arrive at sexual maturity 40-50 days after zoöspore germination in vigorous cultures. As in other members of the order, any cell of the filaments may develop into an oögonium. A terminal cell may simply elongate, but in the case of an intercalary cell a lateral extension develops which is commonly eight or more diameters long (fig. 15, 19, 20). No cross wall is formed, but the nucleus of the cell enlarges greatly and migrates into the extended portion where it takes a position near the center of the oögonium or somewhat nearer the tip. There

is a considerable increase in the number of chromatophores which are mostly peripherally arranged. The center of the oögonium is filled with a large vacuole with numerous strands of cytoplasm in which a number of dark-staining granules are usually visible (fig. 19, 20). The chromatophores may become more elongate in form and in living plants appear paler as in the case of chromatophores in the antheridia, but no evidence was obtained which would indicate that they disintegrate as reported by Kanda (1936, 1938) for several members of the order. Kanda seems to have made his observations from living material only.

McKay (1933) observed a noticeable thickening of the apex of the oögonium before liberation of the egg in the case of Pterygophora californica. Clare and Herbst (1938) report a similar thickening at the tip of the oögonium of Eisenia arborea, but they failed to note an interesting feature usually characteristic of this terminal thickening. I refer to the appearance of numerous papillae, which are frequently rather long and conspicuous (fig. 19, 20) and which form a sort of cap covering the tip of the oögonium. These papillae probably represent local points of gelatinization of the wall. Stained preparations show that, as this cap weakens by gelatinization, it may stretch greatly to form a temporary thin veil over the extruded egg (fig. 21). More frequently it appears to loosen as the result of a transverse rupture, in which case a fragmentary thin cap may be carried outward by the egg as it emerges (fig. 26). Finally, the egg frequently emerges through a more or less longitudinal rupture in the gelatinized papillate cap at the tip of the oögonium

I am of the opinion that extrusion of the egg takes place in a relatively short period of time. Partially extruded eggs are very rarely found in stained preparations showing numerous seemingly mature oögonia and numerous extruded eggs. It seems probable that extrusion is initiated by turgor in the oögonium. Surface tension on the part of the protoplast would seem to account for the final stages in the withdrawal and rounding up of the egg at the tip of the oögonium. A small amount of cytoplasm and a few chromatophores are frequently

left in the oögonium.

Fertilization.—Most investigators have concluded that in the Laminariales the egg is not fertilized until after it is discharged. Stained slides of fertile gametophytes of Eisenia arborea show numerous stages in the development of the oögonium and the subsequent release of the egg and fusion of the antherozoid with the egg. These stages clearly show that the egg is fertilized at the tip of the oögonium after its discharge. When first discharged the egg is a spherical, naked mass of cytoplasm about 18  $\mu$  in diameter with a single large nucleus and numerous chromatophores (fig. 21, 22). The antherozoid appears to establish contact at its anterior end, probably somewhat laterally, where a slender protoplasmic bridge is formed between the

gametes (fig. 22). As this bridge widens, both gametes enlarge noticeably (fig. 23, 24). The increase in size is first evident in the antherozoid and appears to result from a migration of vacuolar sap from the egg into the antherozoid. This may be due to a difference in cytoplasmic density on the part of the protoplasts. The chromatophores of the antherozoid, at first smaller than those of the egg, seem to increase in size along with the increase in volume of the antherozoid. One is inclined to wonder if this considerable increase in volume on the part of the antherozoid is altogether normal. However, I have observed a number of instances of it, and in all cases nuclear stages indicated a normal progress in the process of fertilization. The oöspore averages about one-fourth greater in diameter than the unfertilized egg. The increase in volume appears to be due largely to an intake of water. After the male nucleus passes through the protoplasmic bridge, the antherozoidal bulge is gradually absorbed (fig. 25), and at the time of complete union of the sexual nuclei it is completely obliterated. Early in the process of fusion the egg begins to present a more clear-cut outline, and after fusion is completed, a cell wall is distinguishable.

Williams (1921) reports having observed stages in the union of the sexual nuclei of Laminaria, but his description does not indicate the fate of the cytoplasm and the chromatophores of the antherozoid. The figures and descriptions given by McKay (1933) for Pterygophora indicate that the cytoplasm of the antherozoid fuses with the egg. As shown by the foregoing account, this is definitely

the case in Eisenia arborea.

Early in the process of union of the gametes approximately 15 minute chromosomes are visible in the nucleus of the antherozoid (fig. 23). A similar number appears in the egg nucleus after the male nucleus passes through the protoplasmic bridge between the gametes and approaches the female nucleus (fig. 25). McKay (1933) figures the sexual nuclei of Pterygophora fusing in interphase. My observations show that in Eisenia arborea the chromosome complement of both nuclei is visible at the time of their union to form the zygote nucleus (fig. 25). The zygote nucleus apparently goes into a resting condition before dividing to initiate sporophyte development.

Embryo sporophytes.—In the cultures the eggs almost invariably remain in contact with the tip of the oögonium, where they develop into embryo sporophytes (fig. 15). Clare and Herbst (1938) mention two modes of development of embryo sporophytes. I have examined hundreds of these young sporophytes in various stages of development, and find no reason for distinguishing two methods of development. The short broad sporophyte figured by Clare and Herbst (1938, fig. 5) in my opinion represents a stunted developmental condition. The zygote ordinarily elongates and divides transversely several times to form a monosiphonous filament, which is usually 6–8 cells long and frequently con-

siderably longer, before longitudinal divisions are initiated at the apical end. Rhizoids begin to form from the basal cells when sporophytes are about 4 cells wide (fig. 29). A small amount of cytoplasm containing several chromatophores is frequently present at the tip of young rhizoids as described and figured by Kanda (1938) for Chorda filum (L.) Lam'x., but there are no septa in the rhizoids of Eisenia arborea as reported and figured by Kanda for Chorda. The sporophytes of Eisenia arborea remain monostromatic until many cells wide.

CHROMOSOME NUMBER AND ALTERNATION OF GEN-ERATIONS.—Kylin (1918) first showed for Chorda that meiosis occurs in the first nuclear division of the sporangium. He found a haploid number of 20 chromosomes. Myers (1928), working with Egregia Menziesii, describes reduction division in the first nuclear division of the sporangium and reports a haploid chromosome number of 8. McKay (1933) reports a haploid chromosome number of 13 in the case of Pterygophora californica, with meiosis occurring in connection with the first nuclear division of the sporangium. In Eisenia arborea, as has already been stated, meiosis likewise occurs in the first nuclear division in the sporangium. Fifteen pairs of small chromosomes are visible at diakinesis. I have also been able to distinguish about 15 chromosomes in the nuclei of the vegetative cells of gametophytes (fig. 11). The nucleus of the antherozoid and that of the egg each contain a similar number. Finally, in a two-celled sporophyte embryo approximately 30 minute chromosomes are distinguishable (fig. 28), and a similar number was found to be present in the nuclei of cells from which sporangia are cut off. It is evident, therefore, that in Eisenia arborea a cytological alternation of diploid and haploid phases accompanies the morphological alternation of a macroscopic sporophyte with a microscopic gametophyte and that the life cycle is that of a diplohaplont.

## SUMMARY

The life cycle and the gametophyte plants of Eisenia arborea are essentially like those described for other members of the Laminariales.

Low temperatures are not essential for culturing the sexual plants, which develop and fruit abundantly at ordinary room temperatures of 17-22°C.

Contrary to the findings of certain investigators, the zoöspores are motile and similar to those of other members of the order.

Antheridia are borne in clusters on any cell of the male filaments but do not arise by transformation of the cells of the filaments. The tips of mature oögonia usually become much thickened and covered with numerous papillae.

Antherozoids contain a number of delicate chromatophores rather than one, as generally described for other members of the order. These chromatophores do not disintegrate but are most evident at the time of fertilization.

In fertilization the cytoplasm and chromatophores of the antherozoid are incorporated in the fertilized egg.

The spores and gametophytes of Eisenia are haploid, meiosis occurring during the first nuclear

division of the sporangium. The sporophyte plant is diploid. *Eisenia* is a diplohaplont with a haploid chromosome number of 15.

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# CYTOLOGICAL STUDIES ON THE HEPATICAE. II. THE NUCLEOLUS-CHROMO-SOME IN PALLAVICINIA LYELLII 1

# G. B. Wolcott

Each late telophase nucleus in developing sporophytes of Pallavicinia Lyellii (Hook) Gray contain two nucleoli (rarely one), two hetero-pycnotic chromosomes, and small bits of hetero-pynotic chromatin. These bodies are usually so arranged that one daughter nucleus is a mirror image of the other (fig. 2, 6). The number of such symmetrical nuclei is so large as to exclude any possibility of a chance determination of the location of the nucleoli. These observations have indicated that the nucleolus in Pallavicinia, as in Zea (McClintock, 1934), Vicia (Heitz, 1931), and other plants is associated with a particular chromosome. An investigation of nucleolar origin, behavior, and relationship to chromosomes was undertaken.

MATERIALS AND METHODS.—Pallavicinia was collected near Charlottesville, Virginia. Young sporo-

<sup>1</sup> Received for publication November 7, 1938. This study was made at the University of Virginia and at the Mountain Lake Biological Station. I wish to express my appreciation to Dr. Ivey F. Lewis for supervision of the work.

phytes and the meristematic regions of gametophytes were fixed in the following fluids: Taylor's (McClung, 1937), "Craf" (Randolph, 1935), and Lewitsky's (Lewitsky, 1931). The best results were obtained with Taylor's fixative. The material was sectioned at 10 microns and stained in gentian violet (Smith, 1934) and in gentian violet-safranin (Stockwell, 1934). Wratten filters, appropriate to the stain of the preparations, were used. The slides containing figures 14-16 and 18 were later destained and subjected to the Feulgen reaction. This treatment did not alter the appearance of the images.

Observations.—Early telophase stages (fig. 1) are completely devoid of visible nucleolar material. The nucleolus is first seen as a small globular mass during mid-telophase. Figures 3, 4, and 5 are polar views of sporophytic telophase nuclei during the expansion of the chromosomes to form the resting-stage reticulum. In each nucleus are two small nucleoli attached to, or arising from, the end of a

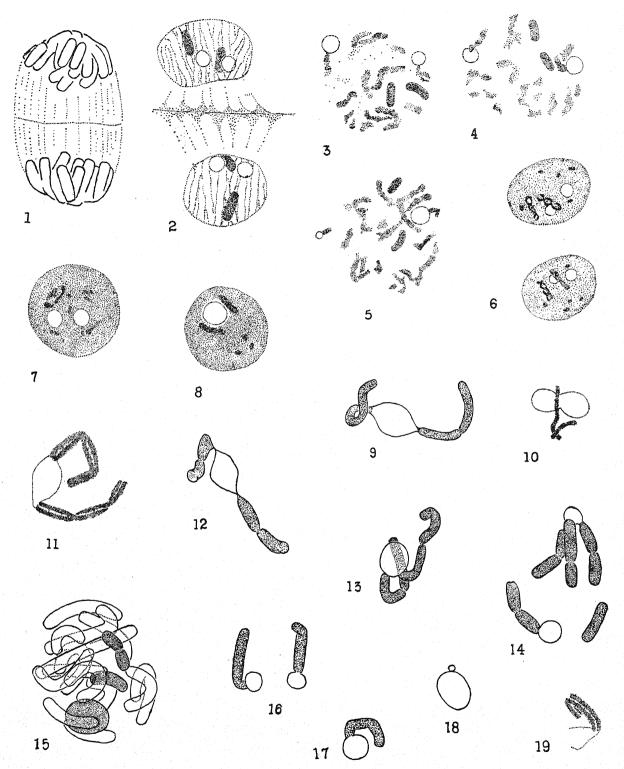


Fig. 1-19. All figures were drawn with the aid of a Zeiss camera lucida and at a magnification of  $3550 \times$ . Figure 17 is from a gametophytic nucleus; all other figures are from sporophytic tissue.—Fig. 1, 2, 6. Daughter nuclei in telophase. Note mirror-image arrangement of elements.—Fig. 3, 4, 5. Polar views of telophase nuclei with nucleoli forming.—Fig. 7. Resting nucleus with two nucleoli.—Fig. 8. Resting nucleus with a single fusion-nucleolus.—Fig. 9, 11, 12, 13. Prophase nucleoli, each with two attached chromosomes.—Fig. 10. Two nucleoli fusing in prophase.—Fig. 14, 16. Unfused nucleoli from late prophase, each with an attached chromosome.—Fig. 15. Late prophase with the full complement of chromosomes and the nucleolus unattached.—Fig. 17. Prophase nucleolus and attached chromosomes.

chromosome. The nucleolus at the left in figure 5 was faintly stained and may be considered as an incipient nucleolus.

As the nuclear membrane is formed during late telophases the nucleoli enlarge. Two daughter nuclei with fully formed membranes but with traces of the spindle still present are shown in figure 2. Figure 6 is a drawing of a later stage in which the spindle has disappeared. At this stage, in resting nuclei (fig. 7), and during early prophases no connection between the nucleolus and any of the chromosomes was visible, though faintly chromatic threads could be seen apparently attached to the nucleolus. The chromosomes are so finely drawn out that reliable interpretations of such a phenomenon are impossible.

As indicated, each nucleolus arises at the end of a chromosome; there are usually two in diploid telophases and never more than one in haplaid nuclei. In resting stages and prophases in sporophytes, however, many nuclei each with a single nucleolus are found (fig. 8). These nucleoli arise from a fusion of the two single nucleoli and are larger. The fusion of two nucleoli in a prophase nucleus is shown in figure 10.

Since, in telophase, each nucleolus is first seen in connection with a single chromosome (fig. 3-5), a fusion nucleolus should have two chromosomes associated with it. Such a condition is shown in figures 9, 11, 12, and 13 from prophase nuclei. The chromosomes are usually attached at opposite sides (or ends) of the nucleolus (fig. 9, 11, 12) but may also be attached adjacently (fig. 13). A few nuclei were found in which nucleoli remained unfused in very late prophase, each retaining its associated chromosome (fig. 14, 16). When the chromosomes have obtained their maximum contraction in late prophases, but have not yet migrated to the equatorial plate, the nucleoli may persist (fig. 15) with no appreciable change in size. The time and manner of complete disappearance of the nucleoli cannot be determined, but no trace of nucleolar material was ever found at metaphase.

The chromosome associated with the nucleolus cannot be identified until mid-prophase (fig. 9). During the later prophase stages, when the chromosomes are more contracted, the chromosome attached to the nucleolus is found to be of medium length, medianly constricted (fig. 14), and will hereafter be referred to as the "n-chromosome." There are a pair of such chromosomes in each sporophytic nucleus and a single one in each gametophytic nucleus (fig. 17).

The nature of the attachment of the n-chromosome to the nucleolus is variable. The end of the chromosome may be flush with the surface of the nucleolus (fig. 11, 13, 16, 19) or may be attenuated (fig. 9, 12). The connection may be severed and the nucleolus lie free (fig. 15). (The hetero-pyc-

notic chromosomes in figures 2, 15 and others will be discussed in a later paper.)

Concurrently with the enlargement of the nucleoli during telophase there is a change from faint pink to a red color in staining reaction with safranin. There is no corresponding loss in chromaticity nor in size in the prophases. An inconstant size difference between nucleoli exists in telophases (fig. 3-6); this is associated with a difference in stain intensity, the smaller nucleolus being less chromatic. All single nucleoli with one chromosome attached to each (fig. 3-5, 14, 16, 17) are spheroidal, as is a fusion nucleolus if the chromosomes are adjacent to each other (fig. 13). When two chromosomes are oppositely attached, the shape assumed by the nucleolus may be amoeboid (fig. 19) or spindle-shaped (fig. 9, 12).

Small buds appear on the nucleolus in prophases (fig. 18). These seem to be the "nucleolosomes" of Sorokin (1929). No connection between these and satellites could be found. The only indication that satellites may exist in this material was the occurrence of apparent satellites on two prophase chromosomes. Despite repeated searchings, these were found only once and cannot be considered significant. The chromosomes bearing these apparent satellites were in the same prophase from which figure 16 was drawn.

Many of the figures referred to do not show complete nuclei, since to draw all chromosomes would obscure the picture.

Discussion.—Although in *Pallavicinia* sporophytes the chromosomes from which the nucleoli arise cannot be identified in telophases, the constant association, during prophases, of the nucleoli with medianly contricted chromosomes of medium size indicates that it is these chromosomes which form the nucleoli in telophase. This conclusion agrees with the work of Heitz (1931), McClintock (1934), Dearing (1934), Kaufmann (1934), and others in assigning nucleolus formation to a particular element in a chromosome complement. There are, however, certain differences in details.

Heitz (1931), using Vicia species as material, found the nucleolar material associated with an achromatic constriction which set off a segment of the chromosome. A similar condition is described by Kaufmann (1934) in Drosophila melanogaster. In Zea Mays the nucleolus has been reported by McClintock (1934) to arise at an achromatic region and to be "organized in the telophase by an enlarged, morphologically distinct, deep staining chromosomal body, which appears at a definite position in one chromosome of the monoploid complement." In the nuclei of Ambystoma tigrinum each nucleolus arises from a pair of subterminal chromomeres in a particular chromosome (Dearing, 1934). However, in Pallavicinia the nucleolus in telophases is at the end of the n-chromosome. A similar telomitic attachment of chromosomes to the nucleolus is reported by Lorbeer (1934) in the liverwort *Frullania*.

The manner of origin of the nucleolus in Pallavicinia is at variance with that reported by Derman (1933) for Callisia, Paeonia, and Pinus, and by Zirkle (1931) for Pinus. These authors describe the formation, among the telophase chromosomes, of nucleolar globules which later fuse to form nucleoli. In Pallavicinia no indication of nucleolar material other than at the ends of chromosomes was found. The formation of nucleoli is not a random appearance of globules but occurs at a single point on a particular chromosome.

## SUMMARY

The nucleolus in Pallavicinia Lyellii arises in late telophase nuclei at the end of a particular chromosome designated the "n-chromosome." There is always a single nucleolus in each haploid nucleus. In each diploid nucleus there are two nucleoli or a single fusion nucleolus.

In late prophases the nucleoli may become detached from the nucleolus-forming chromosomes. During these stages there is no diminution in size nor chromaticity of the nucleoli. No nucleolar material can be identified in metaphases.

The n-chromosome is medianly constricted and is of medium length in respect to the other chromosomes of the haploid complement. The formation of the nucleolus at one point on a certain chromosome is an indication of the spatial organization of that chromosome.

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# NEW SPECIES OF TAPHRINA AND NEW RECORDS FROM WESTERN NORTH AMERICA <sup>1</sup>

# A. J. Mix

IN THE summer of 1937, the writer was privileged to study collections of Taphrina in the herbarium of J. S. Boyce. Many of these collections are from the western United States or adjacent Canada. Also, during the past few years a number of specimens, all of them collected in California, have been received from Lee Bonar, H. N. Hansen, and H. Earl Thomas.<sup>2</sup> Altogether these collections contain a number of interesting new records and three undescribed species.

Taphrina Amelanchieri sp. nov.—Ascis hypophyllis, clavatis aut fere cylindratis, apice rotundatis, 20-30  $\mu$  longis  $\times$  8-13  $\mu$  crassis, cellula basilari fere cylindrata, 8-15  $\mu$  longa  $\times$  6-12  $\mu$  lata; ascosporis saepe octonis, ellipticis aut globosis, 3.5-

<sup>1</sup> Received for publication November 12, 1938.

<sup>2</sup> To these mycologists and to others mentioned in the text grateful acknowledgment is hereby made.

5.5  $\mu \times$  3–5  $\mu$ . "Scopas sagarum" gignens in ramulis Amelanchieri alnifoliae Nutt.

Asci hypophyllous, clavate to nearly cylindric, rounded at apex, 20–30  $\mu$  long  $\times$  8–13  $\mu$  wide; stalk-cells nearly cylindric, 8–15  $\mu$  long  $\times$  6–12  $\mu$  wide; ascospores usually eight in number, elliptic, 3.5–5.5  $\mu$   $\times$  3–5  $\mu$ . Causing witches' brooms on branches of Amelanchier alnifolia Nutt.

Near Frenchtown, Yreka, Siskiyou County, California, Klamath National Forest, E. P. Meinecke and J. S. Boyce.

Type material: Herbarium of J. S. Boyce (Yale University) No. 286.

This fungus is the first species of Taphrina known to occur on a member of the genus Amelanchier, although other species have been described on members of related genera: Crataegus, Pyrus and Sorbus.

In the witches' broom induced by Taphrina Amelanchieri (fig. 1), affected leaves bear the asci over the whole lower surface. Leaves are not noticeably thickened, and under the microscope leaf-cells show little alteration from the normal. Asci of the fungus are shown in figure 2E.

Taphrina Boycei sp. nov.—Ascis hypophyllis, apice rotundatis, in superiore parte fere cylindratis, basim dilatatis,  $30-60~\mu$  longis  $\times$  20-36  $\mu$  crassis, cellula basilari nulla sed asco quoque cum angusto pediculo mycelio conjuncto; ascis juvenilibus guttas pallido-flavas continentibus; ascosporis hyalinis, ellipticis aut globosis,  $4-5~\mu~\chi~3-5~\mu$ . Maculas flavas gignens in foliis Betulae fontinalis Sarg. et B. occidentalis Hook.

Asci hypophyllous, with rounded apex, in upper part approaching cylindric, broadened toward base,  $30-60~\mu$  long  $\times$  20-36  $\mu$  wide, attached to the mycelium by a slender pedicel; young asci containing pale yellow drops; ascospores hyaline, elliptic or globose,  $4-5~\mu~\times~3-5~\mu$ . Causing yellow spots (pale above) on leaves of (1) Betula fontinalis Sarg. and (2) B. occidentalis Hook.

Near Cheekye (1), and at Revelstoke, Big Bend Highway (2), British Columbia, J. S. Boyce and J. L. Mielke.

Type material: Herbarium of J. S. Boyce, No. 1938 (on B. fontinalis) and No. 1963 (on B. occidentalis). Additional material from Malcolm Wil-

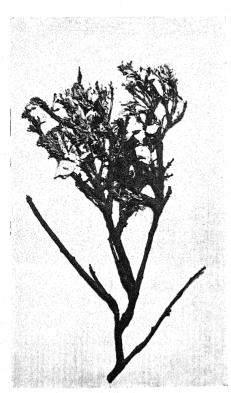


Fig. 1. Witches' broom on Amelanchier alnifolia caused by Taphrina Amelanchieri. (Photograph made from herbarium specimen. Shows some dermestid injury to leaves.) ×1/2.

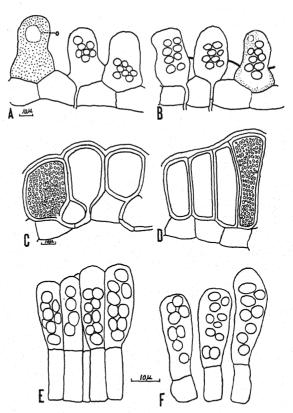


Fig. 2. A. Asci of Taphrina Boycei on Betula occidentalis (one ascus shows a yellowish globule, O, of oily material). B. Asci of the same fungus on B. fontinalis. C. Squarish asci of Taphrina flava, commonly found on the lower epidermis of the host. D. Subcylindric asci of T. flava, more common on the upper epidermis. E. Asci of Taphrina Amelanchieri. F. Asci of Taphrina flectans.

son (University of Edinburgh), New Westminster, British Columbia, July, 1938.

The fungus on Betula fontinalis has somewhat smaller asci than the one on B. occidentalis (fig. 2A, B):  $33-46 \,\mu \times 22-33 \,\mu$  as opposed to  $30-60 \,\mu \times 20-36 \,\mu$ . These slight size-differences might justify distinguishing the fungi on the two hosts as separate varieties, but do not seem sufficient to set them apart as different species.

Taphrina Boycei is obviously closely related to Taphrina flava Farl., which causes yellow spots on the leaves of Betula populifolia Marsh., and B. alba L. var. papyrifera (Marsh.) Spach, but the two fungi show several important differences. Taphrina flava has been studied from Seymour and Earle, Economic Fungi No. 171, and from two collections made by J. H. Faull and kindly loaned by him. Dr. Faull's collections are both on B. populifolia and were made at Kittery Point, Maine, July 7, 1929 (Herbarium of J. H. Faull, No. 9223), and at Chester, Lunenberg County, Nova Scotia, July 22, 1929 (Herb. J. H. F. No. 9252).

The spots caused by Taphrina flava are fairly definite on both surfaces of the leaf, while those

caused by T. Boycei are well defined on the lower surface but appear merely as paler areas in the leaf when viewed from above.

The asci of Taphrina flava are amphigenous. Roundish or squarish, nearly isodiametric asci (fig. 2C) like those figured by Patterson (1895) occur more abundantly on the lower surface of the leaf. On both surfaces, but preponderantly on the upper, occur elongate, nearly cylindric asci (fig. 2D). Asci of each type occur in a close-packed subcuticular layer; and since nearly all the asci mature at the same time, a considerable portion of the cuticle is lifted. The asci of T. Boycei may either be close packed or (by irregular ripening) scattered, and the cuticle may be lifted over a considerable area or may be perforated in places by single asci (fig. 2B). The ascospores of T. flava regularly proceed to budding within the ascus, filling it with minute conidia, before the overlying cuticle is lifted, while the asci of T. Boycei usually contain unbudded ascospores after the asci have burst through the cuticle. Characters common to the two fungi are the yellow color of host-lesions, the presence of globules of pale yellow, oily material in young asci, and the possession of a narrow pedicel connecting each ascus to the intercellular mycelium.

In the material studied the size-limits of the asci of  $Taphrina\ flava$  are greater than those given by Farlow (1883), Sadebeck (1890), and Patterson (1895):  $30-60\ \mu \times 15-30\ \mu$  as opposed to  $38-57\ \mu$ 

imes 20–23  $\mu$ .

Taphrina Pruni-subcordatae causing witches' brooms.—In May, 1938, a witches' broom on Prunus subcordata Benth. was received from H. Earl Thomas. Affected leaves are yellowed, not thickened, and bear asci of a species of Taphrina over the whole lower surface. The asci are clavate to cylindric, rounded or truncate at the apex, and provided with a stalk-cell. Dimensions are: asci, 27-43  $\mu \times 8$ -10  $\mu$ ; stalk-cells, 10-20  $\mu \times 8$ -10  $\mu$ ; spores, 4.5-6  $\mu \times 4$ -4.5  $\mu$ .

That this fungus may be biologically distinct from Taphrina Pruni-subcordatae (Zeller) Mix, which causes "fruit-pockets" on the same host is indicated by the following observations by Thomas. Diseased fruits were not searched for at the place of collection of the witches' brooms (near Elk Creek, California) but were found a few miles away. In another locality (Green Valley) fruits of Prunus subcordata were badly diseased, but no

witches' brooms were to be found.

Morphologically the fungus resembles Taphrina Pruni-subcordatae. Dimensions of the latter as given by Zeller (1927) are: asci, 54- $61~\mu$   $\times$  10- $13~\mu$ ; stalk-cells, 12- $16~\mu$   $\times$  5- $11~\mu$ ; spores, 6- $10~\mu$   $\times$  3.5- $5~\mu$ . In two collections of T. Pruni-subcordatae examined by the writer (Cordelia, California, April 22, 1936; and near Elk Creek, Glenn County, California, May 15-16, 1938; H. Earl Thomas), asci and spores are smaller and stalk- $^3$  Letter from H. Earl Thomas, May 17, 1938.

cells longer than reported by Zeller: asci 34-57  $\mu \times$  8-12  $\mu$ ; stalk-cells 15-30  $\mu \times$  6-10  $\mu$ ; spores 4-7  $\mu \times$  4-6  $\mu$ . It is possible that the variability would be even greater if more collections were examined.

On the basis of morphological similarity, and in spite of an apparent biological distinction, the witches'-broom fungus and the fruit-deforming fungus are considered to be the same: Taphrina Pruni-subcordatae.

In the Boyce herbarium there are specimens (No. 285) of leaf-curl on *Prunus subcordata*, collected near Taylorsville, Plumas County, California, April 23, 1915, by J. S. Boyce, accompanied by the note: "Common, causing witches' brooms. In some cases not deforming." The affected leaves, at least those selected for examination, show small leaf-curl lesions in which no mature asci are to be found. Whether this fungus is the same as the one described above cannot, therefore, be determined.

Taphrina flectans sp. nov.—Ascis hypophyllis, cylindratis aut clavatis, apice rotundatis aut truncatis, in magnitudine et in forma aliquanto variis,  $26-36~\mu$  longis  $\times$   $8-12~\mu$  crassis; cellula basilari  $8-18~\mu$  longa  $\times$   $7-12~\mu$  lata; ascosporis  $4.5-6~\mu$   $\times$   $4-4.5~\mu$ . "Scopas sagarum" gignens et ramulos flectans, in ramis Pruni~emarginatae Walp.

Asci hypophyllous, cylindric or clavate, at apex rounded or truncate, somewhat variable in size and shape,  $26-36 \mu \log \times 8-12 \mu$  broad; stalk-cell  $8-18 \mu \log \times 7-12 \mu$  broad; ascospores  $4.5-6 \mu \times 4-4.5 \mu$ . Causing witches' brooms and bending of twigs on *Prunus emarginata* Walp.

Near Donner Lake, California, July 13, 1938,

H. N. Hansen.

Type material in mycological herbarium, Univer-

sity of Kansas.

Specimens of this witches' broom were received from H. Earl Thomas soon after their collection by Hansen. Besides the usual "brooming" effect, formation of a number of crowded, slender twigs, a negatively geotropic curvature of twigs occurs. (A similar bending of the twigs of a witches' broom is described for *Taphrina Cerasi* (Fkl.) Sadeb. by Ráthay [1881].) Affected leaves are yellowed, unthickened, and bear asci over the whole lower surface.

In appearance and in variability, asci of this fungus (fig. 2F) resemble those of Taphrina Cerasi. Asci of T. Cerasi, according to several authors, are  $35-50~\mu~\times~5-12~\mu$ , stalk-cells  $6-16~\mu~\times~5-9~\mu$ , spores  $6-9~\mu~\times~5-7~\mu$ . Sadebeck (1893), however, states that the asci of this species are the most variable of "any yet studied," and it is the writer's experience that they are indeed variable in size. A number of specimens of T. Cerasi (all on Prunus avium L.) have been studied and the size limits found to be as follows: asci  $19-53~\mu~\times~7-15~\mu$  stalk-cells  $6-23~\mu~\times~4-8~\mu$ ; spores  $4-7~\mu~\times~3-6~\mu$ . It is common to find specimens in which the asci have a length of  $20-30~\mu$ . Since the size-limits of Taphrina flectans lie within those of T.

Cerasi, cross-inoculations involving these fungi and their respective hosts might prove interesting.

A specimen of leaf curl on P. emarginata was received from John Dearness. This was collected at Moscow, Idaho, June 9, 1935. The asci are amphigenous, variable in shape and size, are  $20-36~\mu~\times~8-12~\mu$ ; stalk cells  $8-16~\mu~\times~7-10~\mu$ ; spores  $4.5-6~\mu~\times~4-4.5~\mu$ . More knowledge of this fungus would be desirable, but it is tentatively referred to Taphrina~flectans.

Taphrina unilateralis on Prunus demissa.—Two collections of leaf-curl on Prunus demissa (Nutt.) Walp. have been studied: one from Gulers, Klickitat County, Washington, May 30, 1920, J. S. Boyce; the other from Berkeley, California, May 15, 1937, H. Earl Thomas. The asci are amphigenous, 23–40  $\mu \times$  8–13  $\mu$ ; stalk cells 7–16  $\mu \times$  6–10  $\mu$ ; spores 4–7  $\mu \times$  3.5–4.5  $\mu$ . This fungus was assigned by Atkinson (1894) to Exoascus varius Atk., causing leaf-curl of Prunus serotina Ehrh., "with some

doubt." Atkinson's Exoascus varius was assigned by Giesenhagen (1895) to Taphrina Farlowii Sadeb., deforming fruits of Prunus serotina, but no reasons were given for this reduction to synonymy. It is now apparent that Giesenhagen was right. Material of diseased P. serotina collected April 28, 1938, at Fayetteville, Arkansas, by J. C. Dunegan, and examined by the writer shows one fungus attacking leaves, twigs, and fruits. (When twigs are affected, they show malformations comparable to those caused on Prunus angustifolia Marsh. by Taphrina mirabilis (Atk.) Giesenhagen.) On leaves asci measure 15–30  $\mu$   $\times$  8–13  $\mu$ , stalk-cells 7-13  $\mu \times$  7-8  $\mu$ ; on fruits, asci 13-30  $\mu \times$  7-13  $\mu$ , stalk-cells 7-13  $\mu$   $\times$  7-10  $\mu$ . There is no doubt that the asci on fruits and leaves arise from the same mycelium.

If the leaf-curl fungus on Prunus demissa is to be assigned to a known species, it should be identified with Taphrina unilateralis (Peck) Mix. Measurements of this fungus given by Peck (1898) are stated in decimal fractions of inches. Converted by Saccardo they are: asci 40-52  $\mu$   $\times$  13-16  $\mu$ , stalk cells 13-16  $\mu$  imes 13-16  $\mu$ . These measurements are in error. Examination of Peck's type material (kindly loaned by H. D. House) shows actual dimensions to be: asci 23-33  $\mu$   $\times$  8-13  $\mu$ , stalk cells 8-18  $\mu \times 7$ -10  $\mu$ . The fungus on Prunus demissa is thus seen to be morphologically similar to the type of T. unilateralis, except for somewhat longer asci and narrower stalk cells. Another point of agreement between the two fungi is afforded by the amphigenous hymenium. Peck's "Type 1" of E. unilateralis has epiphyllous asci. His "Type 2," as well as other collections (Raleigh, North Carolina, May 11, 1936, R. F. Poole; Sandwich, Cape Cod, Massachusetts, June 6, 1937, D. H. Linder) examined show asci on both sides of the leaf. From these considerations the leaf-curl fungus on Prunus demissa is identified (for the present, at least) with Taphrina unilateralis.

Taphrina Robinsoniana.—Several specimens of catkin-deformations of Alnus spp. have been studied, as follows: on Alnus rhombifolia Nutt., Putah Creek, Lake County, California, September, 1931, H. E. Parks (communicated by Lee Bonar), asci  $33-53 \mu \times 15-18 \mu$ , stalk-cells  $10-17 \mu \times 10-17 \mu$ ; on A. rubra Bong., Deer Camp Ranger Station, Siskiyou County, California, August 26, 1913, J. S. Boyce, asci 26–40  $\mu \times 13$ –18  $\mu$ , stalk-cells 5–15  $\mu$  $\times$  10-17  $\mu$ ; on A. tenuifolia Nutt.. Glacier National Park, Montana, August, 1938, H. E. Bailev (communicated by Lee Bonar), asci 30-36  $\mu$   $\times$ 10-17  $\mu$ , stalk-cells 7-13  $\mu \times 8$ -13  $\mu$ ; on A. tenuifolia Nutt, Elk River, Clearwater County, Idaho, August, 1928, E. E. Hubert (Herbarium of J. S. Boyce No. 2073), asci 26-33  $\mu \times 10$ -13  $\mu$ , stalkcells 10-13  $\mu \times 10$ -13  $\mu$ .

These fungi may well be considered variants of  $Taphrina\ Robinsonia\ Giesenhag.$  That fungus, deforming catkins of  $Alnus\ incana\ Moench.$ , was described by Giesenhagen (1895) as having asci 29-37  $\mu \times$  6-10  $\mu$ ; stalk-cells 15-17  $\mu \times$  6-10  $\mu$ . Specimens of  $T.\ Robinsoniana\ on\ A.\ incana\ examined by the writer show no significant variations from these dimensions.$ 

Taphrina sp. indet. on Alnus.—Two collections of leaf-curl of Alnus rubra Bong. have been examined: the first made near Hebo, Tillamook County, Oregon, May 9, 1923, by J. S. Boyce; the second near Trinidad, Humboldt County, California, March 24, 1931, by H. E. Parks (communicated by Lee Bonar). Whole leaves are affected, thickened and much enlarged, and changed to a purplish color. The effect is like that produced on leaves of Alnus glutinosa Gaertn. by the European fungus, Taphrina Tosquinetii (Westend.) Tul.

Both surfaces of the leaf are covered with asci. These are irregularly cylindric, seated on the epidermis or slightly inserted, lack a stalk-cell, and measure  $46-76~\mu \times 15-23~\mu$ .

There are two leaf-curling species of Taphrina on Alnus whose asci lack stalk-cells: Taphrina japonica Kusano and T. Alni-japonicae Nishida, both on Alnus japonica Sieb. and Zucc. The former is described as having asci  $60-90~\mu \times 16-25~\mu$ , the latter  $60-80~\mu \times 16-26~\mu$ . Whether these species are really distinct and whether the fungus on Alnus rubra is identifiable with either cannot be told without further study.

Taphrina aurea on Salix laevigata and on Populus spp.—A new host-record is the occurrence of Taphrina aurea Fr. on Salix laevigata Bebb, collected at Mineral, Lassen County, California, September 10, 1911, by E. P. Meinecke (Herb. J. S. Boyce No. 282). The leaf-spots are deep yellow, as is usual for this fungus. The asci are amphigenous, contain numerous minute conidia, and globules of a pale yellow, oily substance. Asci are provided with a stalk-cell which may be short and pointed, but is often long and rhizoidal (even forking) and deeply inserted in the host. The dimensions are: asci  $66-102 \mu \times 20-27 \mu$ ; stalk-cells

20-89  $\mu \times 10$ -17  $\mu$ , narrowing to a point which may be no more than 3  $\mu$  wide.

Taphrina aurea, as it occurs on Populus nigra, usually has a short stalk cell or none. On other species of Populus it is rather variable, and on certain species, as on Populus Fremontii S. Wats., a long, rhizoidal, and occasionally forked stalk-cell may be present. Unless Taphrina aurea is to be split up into several species on different members of the genus Populus, the fungus on Salix laevigata must be placed in that species and not in a new one.

Taphrina aurea is also found on Populus Fremontii S. Wats. and on P. trichocarpa Torr. and Gray in several localities in the Pacific Coast states.

Taphrina spp. on ferns.—Recent records of species that have been previously collected in only one locality are: Taphrina californica Mix on Dryopteris arguta (Kaulf.) Wats., Big Sur River, Carmel, California, June 23, 1938, collected by C. E. Scott (communicated by H. Earl Thomas), the same fungus from San Francisquito Creek, Palo Alto, California, September 25, 1938, collected by R. H. Thompson, and Taphrina Faulliana Mix on Polystichum munitum (Kaulf.) Presl, Lake Cowichan, Vancouver Island, British Columbia, Malcolm Wilson.

### SUMMARY

Through the kindness of various mycologists, it is possible to report on species of *Taphrina* from western North America as follows:

A new species, Taphrina Amelanchieri, causes witches' brooms on Amelanchier alnofolia Nutt.

Another new species, *Taphrina Boycei*, causes leaf-spots on *Betula fontinalis* Sarg. and *B. occidentalis* Hook.

Taphrina Pruni-subcordatae (Zeller) Mix attacks shoots of Prunus subcordata Benth. It is possible that this fungus is biologically distinct from the form causing fruit-pockets on the same host.

A new species, Taphrina flectans, causes witches'

brooms on Prunus emarginata Walp.

The fungus causing leaf-curl of *Prunus demissa* (Nutt.) Walp., assigned by Atkinson to *Taphrina varia* is held to be *Taphrina unilateralis* (Peck) Mix. (*T. varia* is shown to be synonymous with *T. Farlowii* Sadeb.)

Fungi deforming catkins of Alnus rhombifolia Nutt., A. rubra Bong., and A. tenuifolia Nutt. are considered variants of Taphrina Robinsoniana

Giesenhag.

A leaf-curl of Alnus rubra Bong. is caused by an unidentified species of Taphrina showing resemblances to T. japonica Kusano and T. Alni-japonicae Nishida.

Taphrina aurea Fr., besides being well distributed on Populus Fremontii S. Wats., and on P. trichocarpa Torr. and Gray, occurs on a new host, Salix laevigata Bebb.

Fungi found in new localities are: Taphrina californica Mix on Dryopteris arguta (Kaulf.) Wats., and T. Faulliana Mix on Polystichum munitum (Kaulf.) Presl.

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# THE CHROMOSOMES OF RANUNCULUS <sup>1</sup>

L. P. Coonen

THE FAMILY Ranunculaceae, with its 1200 species (Engler, 1936), has furnished much material for cytological study. Particular interest lies in the complex variation of chromosome numbers within the group, as shown especially by Langlet's (1927, 1932) examination of more than 200 species, with haploid numbers in an aneuploid series (including several euploid series) of 6, 7, 8, 9, 12, 14, 15, 16, 18, 20, 21, 24, 28, 29, 30, 32, 35, 42, 64, and 77.

On the basis of chromosome number and morphology, Langlet (1932) establishes two sub-families: Ranunculoideae, with long chromosomes, and Thalictroideae, with short and small chromosomes. These, in turn, are divided into tribes and subtribes, in some instances at great variance with Lotsy's (1911) classification. It is noteworthy that Langlet suggests assigning Glaucidium and Hydrastis (as well as Paeonia) to the Berberidaceae, because only in that family among the Ranales appears the haploid number 5.

Chromosome numbers have been reported for more than 100 species and varieties among the 250 species (Engler, 1936) of the genus Ranunculus. Souèges (1913) reported the haploid number in R. ficaria (=Ficaria ranunculoides Roth) as "about six." No extensive work was done until a decade later (Langlet, 1927, 1932; Larter, 1932; Sorokin, 1924, 1927a, 1927b, 1927c, 1927d, 1929; et al.).

R. acris, because of its small chromosome number, its floral dimorphism, and its dioecism, has been the most extensively studied species. Sorokin (1927a) found a triploid "gynodimorphic" form with a somatic number of 18. The progeny of a cross of triploid  $\times$  diploid (n = 18  $\times$  n = 12) included forms with somatic chromosome numbers of 12, 13, 15, 16, and 17. Apparently normal races with divergent numbers from different geographical locations were reported; 2n = 12 in Russia (Sorokin, 1924); 2n = 14 in the United States (Sorokin, 1927c, 1927d); 2n = 14 + varying numbers of fragments (usually 6) in Sweden (Langlet, 1927); a tetraploid with numbers ranging from 29 to 32 (2n = 28, the extra chromosomes resulting from early splitting) in Russia (Senjaninova, 1926); another tetraploid (2n = 28) in Germany (Langlet, 1932); and a sub-species (R. acris L. subsp. Boreauanus (Jord.) Rouy and Fouc.) with 2n = 16 in France (Hocquette, 1922). Evidence indicates that the typical diploid number for R. acris is 14, this number having been reported by nine investigators (table 1) studying plants from at least seven European countries, the United States, and Japan. Langlet (1927, 1932) found 14 in seven varieties (table 1). Whyte (1929) found the same number in six forms ("normal hermaphrodite," "female,"

<sup>1</sup> Received for publication September 29, 1938. The writer expresses his appreciation to Professor C. E. Allen, who suggested the subject of this research and offered many valued suggestions and criticisms during its progress and in the preparation of this paper.

"male," "abnormal," "minus normal," and "normal X abnormal").

Table 1. List of chromosome numbers in Ranunculus.

(This table follows the procedure of Gaiser (1930): Wherever the authority for a species name had been given by a writer it has been included. Though this may seem unnecessary in most cases, since the chromosome number given for a species name with or without the authority is usually the same, exceptional cases will be noted.)

		Dip-loid	Author
Ranunculus sp.	14		Matsuura & Sutô (1935)
abortivus		16	Langlet (1927)
abortivus L.		16	Sorokin (1929)
abortivus		16	Larter (1932)
abortivus		16	Coonen (present)
aconitifolius		16	Langlet (1927)
aconitifolius L.		16	Larter (1932)
acris L. (Gynodimorphic		10	Larter (1952)
races)		13, 14,	
races		15, 14,	
acris L. (n = $18 \times n = 12$	, ,	12, 13,	Sorokin (1927a)
mer m 12. (n − 10 × n − 12	,	15, 16,	
		13, 10, 17	
agric I (normal V abnor		1,	
acris L. (normal × abnormal)	7		
	7		
acris L. (minus normal)	7		
acris L. (abnormal)	7		Whyte (1929)
acris L. (3)	7		
acris L. (♀)	. 4		
acris L. (Normal herma-	7		
phrodite)	Y .	1.4	, ,
acris L. Hort.	7	14	Coonen (present)
acris L.	7		Sorokin (1927c, 1927d)
acris L.		12	Sorokin (1924,
			1927a)
acris L.		14	Sorokin (1927b,
			1927c)
acris L.		14	Langlet (1927)
acris L.		14	Larter (1932)
acris L.	7	14	Senjaninova (1926)
acris L.		29-32	Senjamnova (1030)
acris var.		28	Langlet (1932)
acris var. albiflora		14	) Langier (1862)
acris var. femina		14	Langlet (1927)
acris var. flor. plen.		14	) ~ ~
acris var. Friesianus		14	Langlet (1932)
acris var. frigidus Regel	7		Matsuura & Sutô (1935)
acris var. Nathorsti		14	)
acris var. prasiniflora		14	Langlet (1932)
acris var. Steveni Regel		14	Miyaji (1927)
acris var. Steveni Regel		14	Langlet (1927)
acris var. Steveni Regel		14	Matsuura & Sutô
			(1935)
acris L. Subsp. Boreauani	18		
(Jord.) Rouy et Fouc.		16	Hocquette (1922)
alpestris		16	
amplexicaulis		16	Langlet (1927)
anemonaefolius		24ª	
apiifolius		48	Langlet (1932)

	Hap-Dip- loid loid	Author		Iap- Dip- oid loid	Author
aquaticus	32	Boecher (1932)	hakkodensis Nakai	16	Matsuura & Sutô (1935)
arvensis	32	Langlet (1927)	hederaceus (= Batrachium		(1933)
arvensis L. asiaticus superbus Hort.	32 16	Larter (1932) Langlet (1927)	hederaceum)	16	Langlet (1927)
asiaticus L.	16	Nakajima (1933)	hederaceus (= Batrachium	10	Dangiet (1331)
	10	Nakajima (1955)	hederaceum)	8 16	Boecher (1932)
asiaticus L. var. Turban	93	T anton (1092)	illyricus	32	Langlet (1927)
Grandiflora	32	Larter (1932)	illyricus L.	32	)
auricomus L.	32	T lot (1090)	Kerneri Freyn.	28	Larter (1932)
auricomus L.	32	Langlet (1932)	Kerneri Freyn.	28	Matsuuro & Sutô
auricomus var.	48 28	Bruun (1932) Larter (1932)	ne. v z rejni		(1935)
auritiifolius Baudotii	16 32	Boecher (1932)	lanuginosus	28	Bruun (1932)
Breyninus	16 32	Langlet (1932)	lanuginosus var. flor. plen.	14	Langlet (1927)
	16	Larter (1932)	lapponicus	16	Langlet (1932)
Broteri Freyn.	16	, ,	Lenormandii Schultz	32	)
bulbosus	16	Langlet (1927)	lingua L.	56	Larter (1932)
bulbosus L.	7	Larter (1932)	y	or 64	
bulbosus L.		Matsuura & Sutô	lingua	128	Langlet (1932)
L. II and T. and an		(1935)	marinus (= Batrachium		Zangier (2007)
bulbosus L. subsp. eu- bulbosus Brig. var.			marinum)	32	Langlet (1927)
	16		millefoliatus	14	)
bulbifer (Jord.) Briq.	16	Hocquette (1922)	monspeliacus	32	Langlet (1932)
bulbosus subsp., eu-bul-			monspessulanus Jord.	32	Coonen (present)
bosus var. bulbifer fa.	10		montanus	16	Langlet (1932)
folis albo maculatis	16	1	muricatus	48	Langlet (1927)
bulbosus var. femina	16	Langlet (1927)	muricatus L.	48	)
bulbosus var. flor. plen.	16	,	Nelsonii Gray	14	Larter (1932)
bullatus	16	Langlet (1932)	nemorosus	16	}
calandrinioides Oliv.	ca. 18	Coonen (present)	nivalis	56	Langlet (1932)
californicus Benth.	28		Nyrranus	16	}
carpaticus	14	Langlet (1927) Lewitsky <sup>b</sup>	ophioglossifolius	16	Langlet (1927)
cassubicus	32		ophioglossifolius Vill.	16	Larter (1932)
cassubicus	32	Langlet (1932)	orientalis	16	Langlet (1932)
caucasicus	16	Langlet (1927)	oxyspermus Ross.	16	Larter (1932)
Chius DC	14	Sorokin (1929)	parviflorus	28	Langlet (1927,
Chius DC Chius DC	14 14	Larter (1932) Langlet (1932)			1932)
			parviflorus	28	Jane (1932)
constantinopolitanus Urv Cymbalaria	. 42 16	Larter (1932)	parviflorus L.	28	Larter (1932)
•	16	Langlet (1927)	paucistamineus (=Batrach	_ ''	
Cymbalaria Pursch.	40	Larter (1932) Langlet (1932)	ium paucistamineus)	16	Langlet (1927)
falcatus fascicularis Muhl.	32		pedatus Walds, and Kit.	28	) ``
ficaria	32	Coonen (present)	peltatus Schrank var.		Larter (1932)
Ficaria L.	32	Langlet (1927)	truncatus Koch.	32	
Ficaria L. var. incumbens			plantifolius	14	Langlet (1927)
Schultz	32	Larter (1932)	$polyan the mus\ L.$	16	)T (1000)
Ficaria L. plant $55/1$	18 +	Larter (1932)	psilostachys Griseb.	32	Larter (1932)
2 12. prant 00/1	2 frag		pygmaeus	16	Langlet (1932)
ficaria subsp. eu-ficaria	2 1142	·')	pyrenaeus	32	Langiet (1952)
Brig.	32	Hocquette (1922)	<i>quelpaertensis</i> Nakai	16	Matsuura & Sutô
ficaria var. flor. plen.	16	` ` ` ` '			(1935)
ficaria var. ochroleuca	32	Langlet (1927)	recurvatus Poir.	32	Coonen (present)
ficaria (Ficaria ranuncu-		)	repens	12	Marchal (1920)
loides Roth)	ca. 6	Souèges (1913)	repens	16	}Bruun (1932)
Ficaria verna	8 16	Negodi <sup>c</sup>	repens	16 32	
ficaria (Ficaria verna)	24	Winkler (1926)	repens	32	Langlet (1927)
Ficaria verna	32	Boecher (1932)	repens	32	Matsuura & Sutô
flabellatus Desf.	32 32	Langlet (1927)			(1935)
flabellatus Desf.	32 32	Matsuura & Sutô	repens L.	32	Larter (1932)
judocomone Dest.	32	(1935)	repens var. typicus Beck.	32	Hocquette (1922)
flabellatus L.	20	(1935) Larter (1932)	repens var. flor. plen.	32	Langlet (1927)
flammula erectus	32 20	Hoquette (1922)	reptans	8	Liehr (1916)
glacialis	32 16	<b>-</b>	reptans	32	Langlet (1927)
Gouanii Willd.	16	Langlet (1932)	rhomboideus Goldie	8 16	Coonen (present)
gramineus L.	16 16	Larter (1932)	rupestris Guss.	28	Larter (1932)
•	· 16	)	sardous	8 16	Langlet (1932)
graminifolius	16	Langlet (1927)	sardous Cr.	16	Larter (1932)

	Hap- Dip- loid loid	Author
sceleratus L.	32	Coonen (present)
scoticus	16	T am -1-+ (1002)
Seguieri	16	Langlet (1932)
septentrionalis Poir.	32	Coonen (present)
serbicus	24 <sup>d</sup>	Langlet (1927)
serbicus Vis.	28	Larter (1932)
Somieri	24 <sup>d</sup>	Langlet (1927)
Sprunerianus Boiss.	16	Larter (1932)
Thora	16	Langlet (1932)
trachy carpus	32	Langlet (1927)
trilobus Desf.	48	Larter (1932)
trilobus	48	T1-+ (100m)
velutinus	14	Langlet (1927)
Vernyi Fr. & Sav.	16	Matsuura & Sutô (1935)
Villarsii	14	Langlet (1932)

<sup>&</sup>lt;sup>a</sup> Corrected to 2n = 28 by Langlet, 1934 (Tischler, 1936).

MATERIALS AND METHODS.—Material of the following species was collected in or near Madison, Wisconsin: R. abortivus L. at Eagle Heights and Observatory Hill; R. acris L. Hort. at the Horticultural Gardens; R. fascicularis Muhl. at the University Arboretum; R. recurvatus Poir. at Poynette and Fern Dell; R. rhomboideus Goldie at Sauk City; R. sceleratus L. at Hope Lake; R. septentrionalis Poir. at Parfrey's Glen.

Identification of the specimens (excepting R. acris) was confirmed by Professor N. C. Fassett. In most instances some material was fixed immediately upon collection; the plants were then potted and kept growing in the greenhouse. Additional fixations were made from them later.

Seed of R. californicus Benth. was procured from Theodore Payne, Los Angeles, California; of R. monspessulanus Jord. and R. calandrinioides Oliv. from H. Correvon et fils, Geneva, Switzerland. After germination the seedlings were potted and grown to the flowering stage in the University greenhouse. Secondary roots were fixed for study.

Seeds of several other European species failed to germinate. Such factors as temperature, light, oxygen, moisture, and permeability of the achenes to water were artificially varied, with no success. Inasmuch as seeds of native species usually responded to ordinary germination methods, it is probable that most of the European ones were no longer viable. Even the percentage of germination in those two species which did produce plants was very low—in the better one only 3.5 per cent; the time required for germination was from 20 to 90 days.

Of the fixing reagents employed, the best general results for somatic divisions were obtained from Flemming's medium solution and from Karpechenko's and Belling's medifications of Navashin's

fixative. Carnoy's acetic acid-alcohol fixative followed by Heidenhain's iron-alum haematoxylin stain gave the best figures of meiotic chromosomes.

For somatic divisions, Smith's (1934) picric-acid modification of Newton's crystal violet-iodine stain was used almost exclusively. Haematoxylin stains the cytoplasm and its included granules, thus making difficult the recognition of satellites and setae. Smith's schedule, however, recommends that slides be left in xylol for at least 10 minutes after differentiation in clove oil; the writer's experience indicates that better results are obtained when this period is reduced to between 5 and 10 minutes. This change does not reduce the durability of the crystal violet stain; 18-months-old slides show no fading.

OBSERVATIONS.—The somatic chromosomes of the species of Ranunculus studied are in general characterized by subterminal, submedian, or median constrictions. Satellites were seen in every species except R. calandrinioides. Since the chromosomes are large, bent, and crowded, it is particularly difficult to find equatorial plates in which they are satisfactorily oriented. In the 28- and 32-chromosome species mitotic equatorial plates present at least 4 or 5 members, or portions of them, which are either perpendicular or oblique to the plate, or are completely or partially obscured by others. In these species specific morphological description of all the chromosomes is therefore difficult or impossible. Only such elements as are especially conspicuous because of their size, constrictions, and satellites can be recognized with certainty from one equatorial plate to another.

Sixteen-chromosome species offer less difficulty. Examination of mitotic equatorial plates often reveals distinct morphological characteristics.

R. abortivus L. 2n = 16.—This number is the same as that reported for the species by Sorokin (1929) in Minnesota, Langlet (1927) in Sweden, and Larter (1932) in England. Sorokin represents the complement by the formula 2 (3V + v + 3J + 1'). Equatorial-plate figures (fig. 4) in the writer's material are in general accord as to chromosome size and form with Sorokin's figures 1 and 2. A pair of chromosomes bearing satellites is conspicuous. Other authors have given only numbers, neither figuring nor describing the chromosomes in this species.

R. rhomboideus Goldie. n = 8, 2n = 16.—The chromosomes (fig. 17, 18) of six equatorial plates, from root tips of three plants, show the characteristics and average lengths indicated in table 2. One chromosome pair (c) is satellited.

The average chromosome diameter is about 0.75  $\mu$ . This is neither stable nor consistent, because of early and varied degrees of spatial separation in equatorial-plate chromosomes. Chromosome length, too, varies somewhat with the stages of mitosis. Pierce (1936) has pointed out that in *Viola* chromosome size and volume vary from cell to cell and (1937) that about a 300 per cent increase in chromosome volume occurs when "excess phosphorus" is

<sup>&</sup>lt;sup>b</sup> Tischler (1936).

<sup>&</sup>lt;sup>e</sup> Tischler (1936).

<sup>&</sup>lt;sup>d</sup> Corrected to 2n = 28 by Langlet, 1934 (Tischler, 1936).

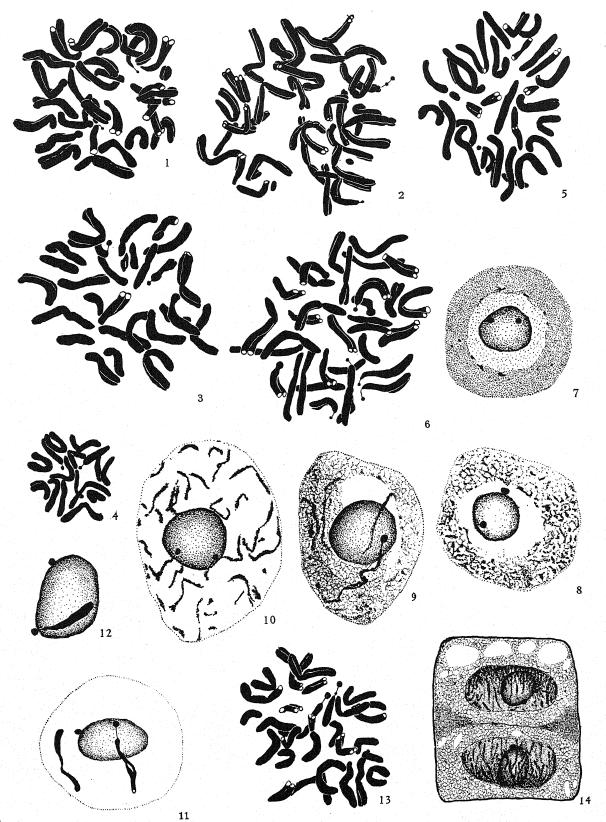


Fig. 1-14. Fig. 1. Ranunculus fascicularis. Polar view of equatorial plate, root tip cell.—Fig. 2. R. monspessulanus. Polar view of equatorial plate, root tip cell; 2 fragments, and tandem-satellite chromosome.—Fig. 3. R. californicus. Polar view of equatorial plate, root tip cell.—Fig. 4. R. abortivus. Polar view of equatorial plate, cell of young petal.

TABLE 2.

Chromo- some	Monobrachial*	Isobrachial	Heterobrachial	Approx. length	Primary constriction
a b	3.0 μ		$3.0 \mu + 1.0 \mu$	4.0 μ 3.0 μ	subterminal
c d	$2.25 \mu + \text{sat.}$	$1.75 \mu + 1.75 \mu$		2.25 μ 3.5 μ	median
e			$2.75\mu + 2.5\mu$	5.25 μ	submedian
g			$3.0 \mu + 2.5 \mu$ $3.0 \mu + 1.75 \mu$	5.5 μ 4.75 μ	submedian subterminal
- h			$3.0~\mu + 3.5~\mu$	6.5 μ	submedian

<sup>\*</sup> Anaphase views indicate that these are telomitic.

used in the soil medium as compared with "minus phosphorus." Evidently variations of similar order may occur in nature.

Variations in size of satellites and in length and diameter of setae have been noted in mitotic equatorial plates, as shown in figures 17, 18, 25, 26, and 27. Tandem satellites (fig. 25) were seen in two instances. An early separation of the halves of the split satellite doubtless explains the appearance of two parallel members on one chromosome (fig. 26).

 $R.\ acris$  L. Hort. 2n=14.—Extensive cytological work has previously been done on this species (table 1). The author's preparations were made from a few buds from a vigorous double-flowered horticultural variety. The cells of young petals showed the diploid number (2n=14). The material was unfavorable for the study of chromosome morphology.

R. calandrinioides Oliv. 2n = ca. 18.—The paucity of available material for study of this species makes cytological determinations uncertain. The only two plants which grew from seed developed very slowly under greenhouse conditions and showed few mitoses in the root tips. Madison's elevation of approximately 900 feet offers conditions vastly different from the plant's native elevation of 8,000 to 10,000 feet (according to Hooker's (1889) citation of Oliver's original description).

When chromosomes are separate they are well defined; constrictions are definite, but the identity of some members of the plate shown in the upper right of figure 33 is questionable. The average diameter (ca.  $1.0 \mu$ ) is greater than in the other species studied, and lengths are considerably less, varying from 1.75 to  $4.75 \mu$ . Fixation was in Belling's modification of Navashin's fixative.

The leaves are strikingly different from those commonly found in the genus, being linear, entire, glabrous, gray-green, and parallel-veined. These divergences, together with the apparent chromosome number (ca. 18) and differences in chromosome size may justify the separation of this species from the genus.

R. sceleratus L. 2n = 32.—Since eight is the commonest basic chromosome number for the genus (table 3), the 16 chromosome pairs present in diakinesis (fig. 16) indicate the possibility of allotetraploidy in this species.

The somatic complement is unique in possessing a pair of large-satellited chromosomes (fig. 13). The satellite diameter  $(0.50 \,\mu$  to  $0.75 \,\mu$ ) is the largest yet figured in the genus. Because of their size the satellites afford a clear contrast to the smaller irregular inclusions of the nucleoplasm and cytoplasm; thus their behavior in mitosis is relatively easily observed. After fixation in Belling's modification of Navashin's fixative and staining in Smith's (1934) crystal violet-iodine, the criteria for favorably stained material are the deep violet of the chromosomes and satellites and the yellow translucency of the nucleoli.

Bodies which may reasonably be assumed to be satellites are very frequently seen lying adjacent to or contiguous with the nucleolus in metabolic cells (fig. 7). Sometimes they are a short distance away from the nucleolus, possibly in consequence of fixation. As many as ten such associations (in as many nuclei) are visible in a single microscopic field. Commonly the bodies in question are recognizable in connection with the nucleoli of a group of three (fig. 15) or four contiguous cells.

In the earliest prophases (fig. 8), when chromonematal clumps are resolving in the nucleoplasm, a clear "Hof" apparently isolates the nucleolus and satellites from other chromophilic substances. A little later (fig. 10) chromonematal strands can be

—Fig. 5. R. recurvatus. Polar view of equatorial plate, root tip cell.—Fig. 6. R. septentrionalis. Polar view of equatorial plate, root tip cell.—Fig. 7-14. R. sceleratus; all from root tip cells.—Fig. 7. Metabolic nucleus; two satellites contiguous with the nucleolus.—Fig. 8. Very early prophase; chromosomal material not visibly associated with satellites.—Fig. 9. Very early prophase; one satellite is connected to chromosomeatal strands.—Fig. 10. Early prophase; chromosomal material becoming organized into stainable chromosomes. Only a small portion of the chromonemata is shown; a few strands are apparently associated with the satellites.—Fig. 11. Prophase; nucleolus associated with two satellite-chromosomes. One chromosome is not connected to satellite (probably disjoined in cutting).—Fig. 12. Late prophase nucleolus, satellites still associated. One chromosome probably disjoined in cutting.—Fig. 13. Polar view of equatorial plate; 2n = 32.—Fig. 14. Telophase; satellites associated with incipient nucleoli.  $\times$  ca. 3250.



Fig. 15-33.—Fig. 15. Ranunculus sceleratus. Group of three metabolic cells from root tip, showing frequency of occurrence of satellites.—Fig. 16. R. sceleratus. Diakinesis, microspore mother cell. One chromosome bears a satellite.—Fig. 17-27. R. rhomboideus.—Fig. 17. Paired homologous chromosomes; root tip cell.—Fig. 18. Polar view of equatorial plate; root tip cell.—Fig. 19. Chromosomes in heterotypic metaphase; one already split.—Fig. 20, 21. Nucleoli and associated satellite chromosomes in prophase of sporogenous cells. Chromosome on left in figure 20 lies below the nucleolus.—Fig. 22, 23. Nucleoli and associated satellite chromosomes in prophases; root tip cells.—Fig. 24. Satellited meiotic chromosomes, heterotypic anaphases in three pollen mother cells, all from one bud.—Fig. 25. Chromosome with tandem satellites, and its homologue with a single satellite; root tip cell.—Fig. 26. Homologous somatic chromosomes; one bearing pair of satellites, or a precociously split satellite; root tip cell.—Fig. 27. Homologous chromosomes, showing variation in seta length; root tip cell. (Cf. c, fig. 16.)—Fig. 28-30. Chromosomes from 3 equatorial plates of root tip cells in R. fascicularis, showing variation in setae and satellites.—Fig. 31. Tandem-satellited chromosome, root tip cell, R. septentrionalis.—Fig. 32. Nucleoli with associated satellites, root tip cells, R. californicus. (Semi-diagrammatic.)—Fig. 33. R. calandrinioides. Polar view of equatorial plate, root tip cell. All × ca. 3250 except fig. 15 (×1850).

seen directed toward the satellites, apparently touching them; visible setal attachment is sometimes certain (fig. 9). When the chromonemata and matrices contract to deeply staining chromosomes in the median and late prophases (fig. 11,

12), the definite setal attachment is more often observed. In these stages the satellite, seta, and proximal end of the chromosome usually lie on the surface of the nucleolus.

At the metaphases the halves of the split satellite,

associated seta, and chromosome separate—those of the satellite and seta sometimes precociously. Excessive destaining at this stage renders the chromosome delicately purple and translucent, while the satellite remains a deep violet.

In the telophases the satellites persist after the apparent dispersion of the organized chromosomes and can be seen on the surfaces of the incipient nucleoli (fig. 14).

During the entire mitotic cycle the satellites undergo no appreciable change in morphology nor in their affinity for the crystal violet stain.

R. Monspessulanus Jord. 2n = 32.—This European species, also a tetraploid, grew slowly under greenhouse conditions, and its root tips showed relatively few mitoses. Equatorial plates ordinarily showed 32 somatic chromosomes, two bearing single satellites; in one instance an equatorial-plate chromosome bore tandem satellites (fig. 2). One root tip showed two fragments (fig. 2). Since some equatorial plates definitely showed the fragments lying about midway between the surfaces of the  $10 \mu$  section, they could not be attributed to sectioning.

R. californicus Benth. 2n = 28.—This tetraploid possesses a pair of chromosomes with satellites of unequal size (fig. 3). Nucleoli of resting nuclei (fig. 32) show two attached bodies, likewise of unequal size.

R. fascicularis Muhl. 2n = 32.—Two small satellites are found in this tetraploid species (fig. 28, 30). Variations in size of satellites and in length of setae have been noted. Tandem satellites were found in a single instance (fig. 29).

R. recurvatus Poir. 2n = 32.—Two satellites have been seen in many cases. In each instance the chromosomes were too crowded to distinguish all members of the equatorial plate. Figure 5 shows the tetraploid number, with but one satellite; its setal connection could not be determined.

R. septentrionalis Poir. 2n = 32.—Varying numbers of satellites have been seen in this tetraploid species; the maximum, 5, are shown in figure 6. Because of the large chromosome number and of the size and orientation of the chromosomes it has been impossible to determine whether or not this is a constant number. One equatorial plate showed tandem satellites on one chromosome.

Discussion.—Chromosome numbers of 95 forms of 85 species of Ranunculus thus far studied, including those reported in the present work, are summarized in table 3 and figure 34. In case an author has expressed uncertainty or has studied inconstant aberrant forms, his report has been omitted.

The basic chromosome number 6 has been reported but once in R. acris (table 1). A race, 2n=24, of R. Ficaria is assumed to be a triploid with the basic number 8, rather than a tetraploid with the basic number 6. The diploid numbers 16 and 32, and no other multiples of 6, have been reported in the species.

As 8 is the most frequent basic chromosome num-

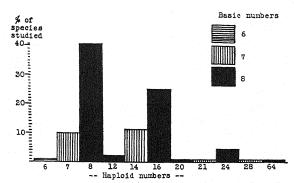


Fig. 34. Graph showing distribution of haploid and basic chromosome numbers in Ranunculus thus far studied.

ber (table 3), others (6 and 7) are possibly derived from it. Sorokin (1927b) compared the chromosome complement of a 14-chromosome race of R. acris with that of a 12-chromosome race and found the latter to differ from the former in the absence of a pair of large chromosomes (9.4  $\mu$  in length). All pairs of chromosomes in the 14-chromosome race differ from one another; it is not likely that through trisome- or tetrasome-formation it had been recently derived from the 12-chromosome race. Chromosome loss may conceivably have been the cause of derivation of the basic number 7 from 8. However, there is no evidence to support this possibility; gene mutations and structural differentiation in the course of evolution may have altered individual chromosome character so greatly that morphological comparison is not a valid criterion.

TABLE 3.

Number of species	Percentage of total	
39	41.05+	
24		
	11.58—	
10	10.53—	
4	4.21 +	
2	2.10+	
1	1.05 +	
1	1.05+	
1	1.05 +	
1	1.05 +	
1	1.05+	
Number of	Percentage	
species	of total	
65	74.71+	
21	24.14-	
1	1.15	
	species  39 24 11 10 4 2 1 1 1 1 1 Number of species  65 21	species         of total           39         41.05+           24         25.26+           11         11.58-           10         10.53-           4         4.21+           2         2.10+           1         1.05+           1         1.05+           1         1.05+           1         1.05+           1         1.05+           1         1.05+           1         1.05+           1         1.05+           2         74.71+           21         24.14-

Both allopolyploids and autopolyploids probably exist in the genus. Determinations of either type in the few species attempted are uncertain; no significant cytogenetic work has been done.

Fragments have been reported in several species of the genus. Jane (1932) found a pair of frag-

ments in "some metaphases" of R. parviflorus. Larter (1932) reported an extra pair of small chromosomes in one plant of R. Ficaria. Langlet (1927) saw varying numbers of "e-Chromosomen" (usually 6) in a root tip of R. acris growing wild in Sweden; he attributed their origin to complete constriction (Einschnürung) in the positions of subterminal constrictions in normal chromosomes. The origin and significance of the fragments in R. monspessulanus (fig. 2) are quite uncertain.

A few tetraploid cells (with about 64 chromosomes) have been found by the writer in root tips of R. monspessulanus and R. fascicularis. A similar occurrence is reported by Larter (1932) in R. sardous and R. Ficaria. He found tetraploid strains in the latter species and suggested that they may have originated from such cells of a diploid

strain.

Since Navashin (1912) reported the satellites of prophase and metaphase chromosomes of Galtonia candicans, and similar bodies attached to the nucleoli of resting cells, various similar associations have been reported in other organisms. Heitz (1931) postulated the ubiquity of satellites. Kuhn (1928) has listed 130 species, representing 38 genera, known to possess satellite chromosomes. Work since then has shown satellites to be even more prevalent. Babcock and Cameron (1934) reported them in 108 Crepis species; Resende (1937) found them in 185 species (the majority members of the section Aloinae of the Liliaceae); they have been reported in almost every genus of the Ranunculaceae.

As early as 1911 Stevens (1911) figured a pair of satellite chromosomes, which she called "heterochromosomes," in the mosquito. Since 1933 satellites have been reported in five other animals: Drosophila, Bibio, Amblystoma, Opalina (cited by Resende (1937), p. 759), and Salmo (Prokofieva, 1934).

In the present work no setal connection was determined between nucleolus and satellite in resting somatic nuclei, as figured by Sorokin (1924, 1929) and Hasse-Bessell (1928). Contiguity of nucleolus and satellite was noted in R. rhomboideus (fig. 20-23), R. californicus (fig. 32), and R. sceleratus

(fig. 7-12, 14).

Among the various reported opinions as to the function and nature of satellites are the following: Navashin (1927) considered that a satellite is picked up by the chromosome during the prophases and that, as a terminal chromomere, it plays some rôle in the attachment of the spindle fiber to the chromosome. De Mol (1927) considered a similar structure to be a nucleolar globule not taken up by the chromosome. Delaunay (1915) considered satellites to be concerned with chromosome contraction. Darlington (1937) held a satellite to be a short chromosome arm unique only in its formation by a broad constriction; Heitz (1931) thought satellites concerned with nucleolus-formation. But McClintock (1934) finds the nucleolus formed in connec-

tion not with a satellite but with a special chromatic portion of the chromosome near the seta.

The writer's observations of the satellite of R. sceleratus and its behavior in mitosis suggest the following: (1) that it is present in all mitotic stages, having been seen in all but early telophase, at which time conditions are most unfavorable for its observation; (2) that it has an affinity for the nucleolus, satellite and nucleolus having been seen in apparent contact whenever both bodies are present; (3) that its chromophilic qualities are different from those of both nucleolus and chromosome body; in destaining it retains a deep crystal violet stain after equatorial-plate chromosomes have been almost, and the nucleoli entirely, destained; (4) that it is apparently morphologically constant; (5) that it is always associated with the chromosome, whether the chromosome is or is not recognizable as a stainable entity; soon after the nuclear material appears as small irregular stainable clumps of chromatin in the early prophases the satellite has been seen attached to ill-defined chromonematal strands (fig. 9, 10).

Variation in size of satellites or in length of setae has been noted in certain of the species here studied (fig. 2, 18, 27, 28, 30); but since such delicate structures and their observation may be influenced by any one, or by any combination, of several variable factors (mitotic stage of chromosome, reaction to fixative, reaction to stain, and orientation of the observed chromosome), no explanation is attempted.

Tandem satellites have been observed in rare instances (fig. 2, 25, 29, 31). Taylor (1926) has found similar tandem satellites in the root tips of a single Allium plant, the homologous chromosome in this case probably lacking a satellite. Meurman (1929) figures four chromosomes of Aucuba japonica with tandem satellites, definitely separated by threads. He evidently considers them to be small constricted portions, for he does not refer to them as satellites.

The origin of tandem satellites in R. fascicularis and R. septentrionalis, the homologue apparently bearing none, may be attributed to faulty disjunction in the metaphases. But in case the homologue does bear a single satellite (fig. 2, 25), the origin of the double structure would necessarily be referred to an ancestral plant, some of whose gametes contained tandem satellites and others none. A gamete of the former would have fused with a gamete having a single-satellite chromosome to produce the condition here found.

## SUMMARY

Chromosome numbers were determined as follows: Ranunculus abortivus L., 2n = 16; R. rhomboideus Goldie., n = 8, 2n = 16; R. acris L. Hort., 2n = 14; R. calandrinioides Oliv., 2n = ca. 18; R. sceleratus L., 2n = 32; R. monspessulanus Jord., 2n = 32; R. californicus Benth., 2n = 28; R. fascicularis Muhl., 2n = 32; R. recurvatus Poir., 2n = 32; R. septentrionalis Poir., 2n = 32.

The average lengths of the respective somatic chromosomes of R. rhomboideus are: 2.25  $\mu$ ; 3.0  $\mu$ ;  $3.5 \mu$ ;  $4.0 \mu$ ;  $4.75 \mu$ ;  $5.25 \mu$ ;  $5.5 \mu$ ;  $6.5 \mu$ .

Divergences in chromosome morphology and number, as well as in vegetative characteristics, may justify the removal of R. calandrinioides from the

Satellites were observed in all species studied excepting R. calandrinioides.

Two large satellites were seen at all mitotic stages, except early telophases, in R. sceleratus. Their chromophilic qualities are different from those of both nucleolus and chromosome body. They are apparently in contact with the nucleolus whenever it is present, and are always associated with their respective chromosomes.

Tandem satellites were found in isolated cases in R. monspessulanus, R. rhomboideus, R. fascicularis, and R. septentrionalis. Variations in satellites and setae were noted in some species; these may be due to variable factors in the preparation and observation of material.

A few tetraploid cells were observed in root tips of R. monspessulanus and R. fascicularis.

A pair of fragments was found in the equatorial plates in one root of R. monspessulanus.

Of the 95 forms of 85 species thus far studied in the genus, 74.71 per cent have the basic number 8; 24.14 per cent, 7; and 1.15 per cent, 6. Basic numbers 6 and 7 are probably derived from 8.

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# POTENTIALLY UNLIMITED GROWTH OF EXCISED PLANT CALLUS IN AN ARTIFICIAL NUTRIENT <sup>1</sup>

Philip R. White

ONE of the ultimate objects of the cultivation of isolated plant or animal organs or tissues has always been, as Haberlandt (1902) long ago pointed out, the investigation of problems concerning the metabolism of the individual cell and its relation to neighboring cells. This object can be fully attained only if the cell can be isolated from its fellows, either anatomically or physiologically. Haberlandt and his immediate pupils sought to isolate cells anatomically. But the same end can be attained approximately if a completely undifferentiated mass of tissue can be grown in which all cells are alike and hence exert like influences on one another. It is for this reason that an "undifferentiated" state has come to be considered one of the irreducible characteristics of a "tissue culture." There is, however, another irreducible characteristic of a true "tissue culture" which must be demonstrated if the metabolism of the cells is to be considered normal. That is unlimited capacity for growth. This question has been repeatedly discussed elsewhere (White, 1931, 1936).

As was pointed out in earlier papers (White, 1934, 1936), the root cultures maintained in this laboratory for the past six years satisfy the second requirement, of unlimited capacity for growth, but are by no means undifferentiated. Gautheret's cultures of excised cambium (1934, 1935, 1938a, 1938b), Nobécourt's carrot root cultures (1937, 1938), and Bonner's pea-pod cultures (1936), on the other hand, are relatively undifferentiated and have the appearance of tissue cultures. Gautheret has grown cambium cultures for more than a year, through five or six passages, thus furnishing evidence of a possible capacity for unlimited growth. It seems probable that he has, therefore, fulfilled both these requirements and is to be credited with the first true plant tissue cultures. His observations, however, do leave some doubt on this score. The tissue fragments used in his cultures were relatively large, one to two centimeters in diameter, and contained considerable stored material. The growth rates reported were so low that transfers could be made only once every 4 to 6 weeks. The cultures may have survived at the expense of the stored nutrients within the explant and may not be capable of unlimited growth. Nobécourt's cultures likewise grew slowly and were of relatively brief duration. Bonner's results were still less satisfactory. A more adequate demonstration is needed than that furnished by the data of these authors.

I have for some time been interested in a hybrid tobacco resulting from the cross Nicotiana glauca  $\mathcal{P}$   $\times$  N. langsdorffii  $\mathcal{S}$  (Kostoff, 1930; Levine, 1937). This hybrid has an unusual capacity for prolifera-

tion, producing calluses and galls at any point on stem or leaf which has been mechanically abraded. Callus tissue may also develop in the absence of obvious wounding. It seemed possible that tissues of such a plant might be especially favorable for cultivation in vitro. This paper reports the results of an investigation of this possibility.

The nutrient used was the same as that employed for cultures of isolated roots, consisting of a modified Uspenski solution, 2 per cent sucrose and 0.01 per cent of an extract of dried brewer's yeast (White, 1934). It early became evident that most callus tissues would not float on the surface of the nutrient as do roots, so that their oxygen supply

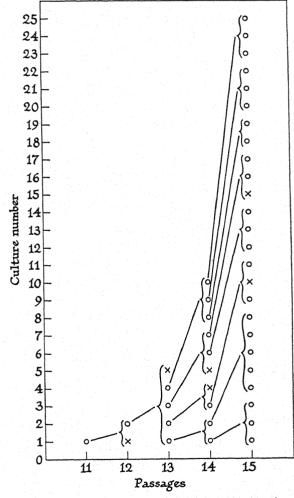


Fig. 1. Diagrammatic representation of the genealogy of the 25 cultures of passage 15, showing their derivation from culture No. 2, of passage 12. Cultures marked with a cross (x) were contaminated with molds or bacteria.

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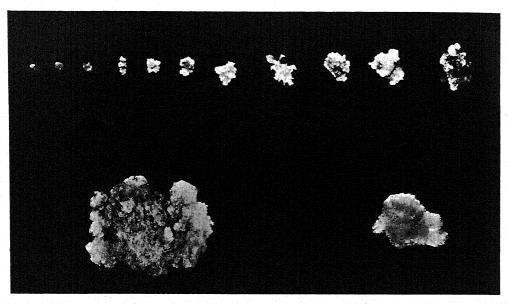


Fig. 2-4.—Fig. 2 (above). A series of cultures of excised hybrid tobacco callus cultivated in vitro. The cultures vary in age from 0 (extreme left) to 10 weeks (extreme right). ×1.7.—Fig. 3 (lower right). Detail of a tissue fragment similar to that shown at the left in figure 2. The surface is irregular, but there is no evidence of differentiation. ×20.—Fig. 4 (lower left). Detail of a 20-weeks-old culture. There is still no macroscopic evidence of differentiation. The tissue mass is so friable as to be easily broken up with a needle. ×2.3. (Photographs by J. A. Carlile.)

might be expected to be inadequate in a liquid nutrient (Zimmerman, 1930; Gautheret, 1935; see also various papers by W. A. Cannon and by D. R. Hoagland and associates). To obviate this difficulty, 0.5 per cent of thoroughly washed agar was added to the above nutrient to make a semi-solid medium. Young stems of the plants were stripped of their leaves. In the laboratory they were broken 4 to 5 cm. back of the tips, where the tissues were brittle enough to break without tearing. Clean. aseptic surfaces were thus exposed. With a sterile scalpel, cones of tissue were removed from the exposed surfaces, the tissue masses consisting of medulla and procambial strands. The excised masses were always less than 5 mm. in greatest diameter. They were placed on nutrient agar in either 125 ml. Erlenmeyer flasks or 40 ml. Pyrex test tubes and set aside in diffuse daylight.

Where such fragments contained only medullary parenchyma, no growth occurred, and the tissues soon turned brown. Wherever procambial strands were present, however, proliferation from these regions began almost immediately. Irregular protuberant masses of callus were formed, which early took on a bright green color. After from one to two weeks, these masses were cut away from the original explant and transferred to fresh nutrient. The cultures were divided and transferred to fresh nutrient at biweekly intervals through 10 passages. Five rapidly growing cultures were then selected for more detailed study and the remainder dis-

<sup>2</sup> This color was retained in cultures that were subcultured at frequent intervals but disappeared in older cultures, without causing any concomitant reduction in growth rate. carded. These 5 were divided into 25 fragments, each less than 2 mm. in diameter. thenceforth were at weekly intervals, and each culture was regularly divided into as many pieces of 1 mm. or less diameter as possible, 25 cultures being retained in each passage for the next 10 passages. From figure 1 it is clear that by the 15th passage all 25 cultures were derived from culture No. 1 of passage 11 and culture No. 2 of passage 12. The tissue fragment used as No. 2, passage 12, had therefore increased 25-fold in 3 passages. The detailed record shows that each fragment had increased on an average about 3-fold per passage of 7 days, or about 50 per cent per culture per dayan increment rate which compares favorably with that observed in isolated roots. At the end of the 20th passage, the number of cultures was reduced to 10 to conserve space. These cultures had been maintained through 40 passages on November 3, 1938, and are still in excellent condition at the time of writing this paper.

An increase of 3-fold per passage for 40 passages would represent an increase of  $3^{40} = \text{ca. } 1.2 \times 10^{19}$ , or a dilution of any material contained in the original explant to a mass of the order of  $10^{-19}$  times its original mass in each culture. It is not to be supposed that any material could be a growth-limiting factor at such a dilution. These cultures, therefore, appear to satisfy the second requisite of a tissue culture, unlimited capacity for growth.

Figure 2 shows a series of cultures prepared by setting aside one culture each week for the ten weeks involved in passages 11 to 20. The oldest piece (extreme right) had thus been undivided for

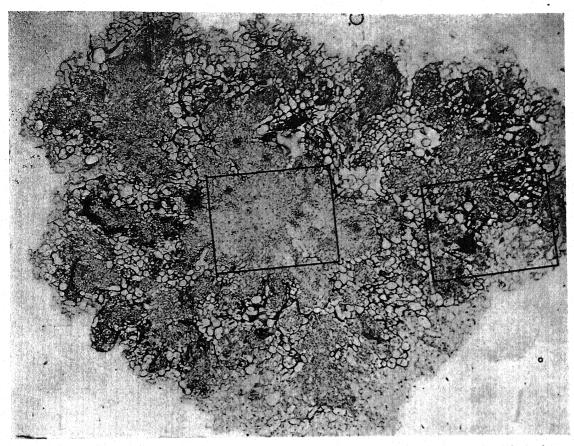
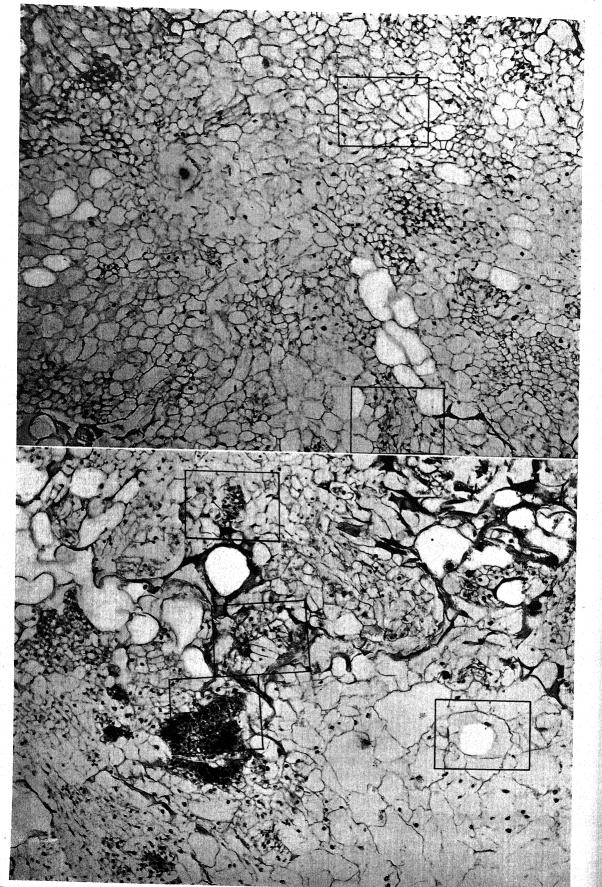


Fig. 5. Transverse section through an 8-weeks-old culture similar to that shown third from the right in figure 2. The central area (see fig. 6) is relatively uniform, while the marginal area (see fig. 7) is less compact and is made up of irregular masses of tissue which might be mistaken for organized growing points or leaves. ×30. (Photograph by J. A. Carlile.)

10 weeks, while the youngest (extreme left) was photographed at the time of subculturing and shows the size of piece used in starting each subculture. Figure 3 shows the detailed appearance of such a culture at the beginning, and figure 4 a similar culture after 20 weeks' growth. There is little if any macroscopic evidence of differentiation. The microscopic structure is in keeping with this appearance. Figures 5 to 13 were all taken from a single transverse section through the center of a culture which had been maintained for 8 weeks without being divided. In general appearance the section shows a central core of tissue resembling medulla (fig. 5, 6) surrounded by an irregular corona of less compact masses (fig. 5, 7). central core is made up of relatively uniform, moderately large cells with scattered areas of what may have been meristematic tissue and occasional groups of scalariform cells suggesting differentiation into xylem (fig. 6, 8, 9). These xylem elements are isolated and obviously incapable of normal function, since they do not extend for any considerable distances through the mass. The younger, peripheral regions show a much wider variation in cell type. The meristematic areas are more frequent

(fig. 7, 10, 11) and are obviously functional. Growth thus occurred by means of "growing points" rather than cambium, but these growing regions are not identifiable as "stem" or "root." Growth was random, neither polarized nor resulting in oriented differentiation. The meristematic cells evidently divide for a time, then enlarge (fig. 7, 11, 13), form irregular bands of "giant cells" (fig. 7, 12), and these finally either become thickened (suberized?) (fig. 5, 7) or else are crushed by the new growth. The outermost portions of the tissue mass are usually made up of large, thin-walled cells (fig. 7, 12). The cells throughout the culture contain considerable quantities of starch, although they are nearly if not completely devoid of chlorophyll (fig. 9, 10, 11, 12, 13). Nowhere were the loose "pleurococcoid" growths figured by Gautheret (1934, 1935, 1938b) nor the arcuate cells of Nobécourt observed (Nobécourt, 1937-compare with the sloughed cortical cells of wheat-root cultures. White, 1932, and those observer by Scheitterer, 1931).

These cultures, then, show a behavior similar to that of attached callus but do not undergo anything like the same degree of differentiation (Le-



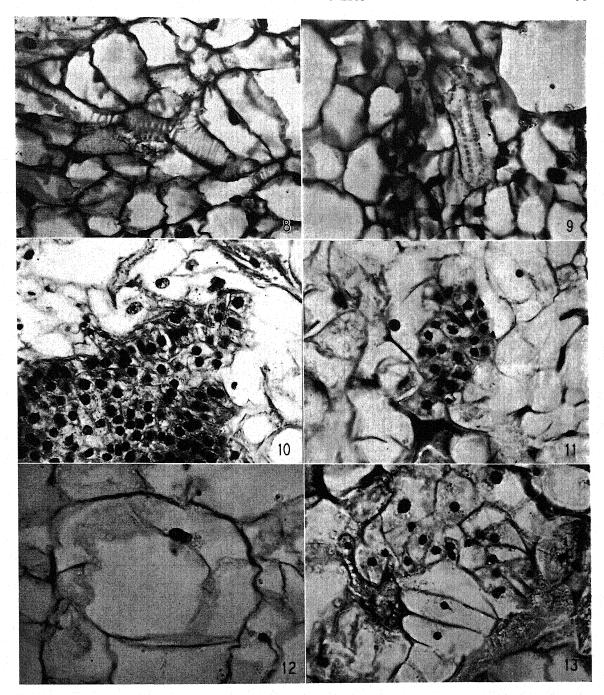


Fig. 8-13.—Fig. 8. Detail of a band of scalariform cells from the upper part of figure 6.  $\times 435$ .—Fig. 9. Detail of scalariform cells from the lower part of figure 6.  $\times 435$ .—Fig. 10. Large meristematic area from the center of figure 7.  $\times 435$ .—Fig. 11. Small meristematic area from the upper part of figure 7. Compare the size of these cells with those in figures 12 and 13.  $\times 435$ .—Fig. 12. Detail of a "giant cell" from the right hand part of figure 7. Compare in size with the meristematic cells of figures 10 and 11.  $\times 435$ .—Fig. 13. Detail of a group of cells in figure 7, center, which are obviously derived by division from a small group, possibly 3, of meristematic cells.  $\times 435$ . (Photographs by J. A. Carlile.)

Fig. 6 (above). Detail of the central part of figure 5. The cells of this region are of relatively uniform size, thin-walled, parenchymatous in nature except for scattered denser groups of semi-meristematic cells and occasional scalariform cells (see fig. 8 and 9). ×138.

Fig. 7 (below). Detail of a marginal area from figure 5. There are scattered clumps of meristematic cells (see fig. 10, 11) with bands of necrotic tissue, areas of parenchymatous "giant cells," and areas of intermediate type. What at low magnification (fig. 5) appeared to be organized stem growing points appear at higher magnification to be quite unorganized. ×138. (Photographs by J. A. Carlile.)

vine, 1937). It is not possible to identify phloem, cambium, pheloderm, sclerenchyma, nor any other normal cell type except parenchyma, meristem, and an occasional isolated scalariform cell. The cultures, while not quite unorganized, are at least quite disorganized. The tissues are not pith, wood, bark, nor even typical callus. These are not organ cultures. While not strictly comparable to the "pure line" cultures of the animal tissue culturists, they do represent a very close approach thereto. Although all the cells are not quite alike, these masses represent cultures of cells characterized by a very simple and primitive type of growth. It is believed that we have here at last a very near approach to a true plant tissue culture, which should provide us with a new means of approach to the problems of the physiology of the spermatophyte cell.3

<sup>3</sup> Since preparing this paper for publication, similar cultures have been successfully established from Nicotiana tabacum L., Lycopersicon esculentum Mill., and Beta vulgaris L., and are now (January 11, 1939) in the sixth passage. This capacity is, therefore, not a special characteristic of the N. glanca × N. langsdorffii hybrid, but a more general characteristic of normal plants.

## SUMMARY

Excised callus obtained from proliferating procambial tissue of a hybrid Nicotiana (N. glauca X N. langsdorffii) has been maintained in culture in an environmental complex and nutrient similar to those earlier developed for cultivation of excised roots, through 40 passages of one week duration Cultures regularly increased about 3-fold in volume each week, giving a total theoretical increment of  $3^{40} = ca$ .  $10^{19}$ . The conditions can, therefore, be considered adequate for unlimited growth of this material. These cultures show no evidence of differentiation or polarity except for an occasional scalariform cell. Being undifferentiated yet capable of unlimited growth, they appear to satisfy the two main requirements for a true "tissue culture."

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## ARTIFICIAL INDUCTION OF POLYPLOIDY IN ALFALFA 1

D. C. Cooper

RANDOLPH (1932) devised a method for the artificial induction of polyploidy in maize wherein he subjected the ear shoot to temperatures of 30° to 45°C. for half-hour intervals at the time of the first division of the zygote. Since then Atwood (1936) and Muntzing, et al. (1936) have made

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use of a similar technique and induced polyploidy in sweet clover and barley, respectively. Dorsey (1936) obtained tetraploid plants of wheat and rye by subjecting the entire mother plant to a temperature of approximately 43°C. for 20–30 minutes at the time of division of the zygote. Peto (1936) kept barley seedlings at a temperature of 35°C. for a period of seven days following germination, after which time they were carried to maturity under normal growth conditions. One plant produced a spike bearing both diploid and tetraploid florets.

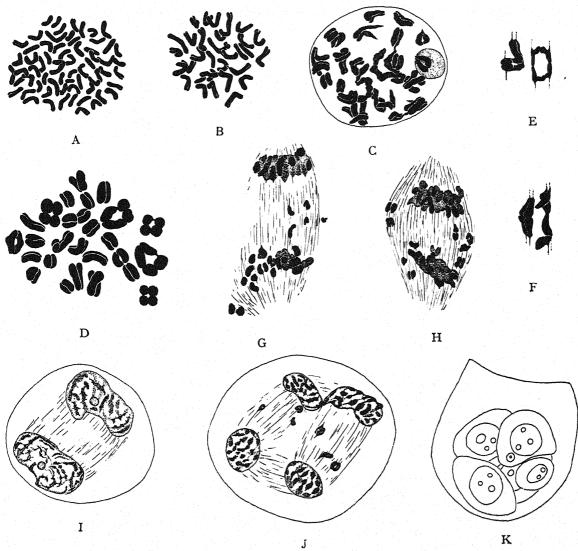


Fig. 1.—A. Somatic chromosomes of tetraploid plant. 2n=64.—B. Somatic chromosomes of a sister diploid plant. 2n=32.—C. Diakinesis in tetraploid plant showing 4 quadrivalents and 24 bivalents.—D. Polar view of a first meiotic equatorial plate with 4 quadrivalents and 22 bivalents.—E and F. Quadrivalents from first meiotic equatorial plates.—G and H. Late anaphases showing lagging chromosomes.—I. Interphase.—J. Second meiotic telophase with lagging chromosomes left in cytoplasm.—K. Spores derived from a single microspore mother cell. One contains two nuclei. Magnification: A-J,  $\times 2560$ ; K,  $\times 1100$ .

During the spring of 1936 an attempt was made to induce polyploidy in alfalfa (Medicago sativa L.) in a similar manner. Fertilization takes place in this plant between 24 and 28 hours after the tripping of the flower, and the division of the zygote nucleus occurs three to four hours thereafter. The apical cell of the resulting pro-embryo continues to divide so that ultimately the pro-embryo consists

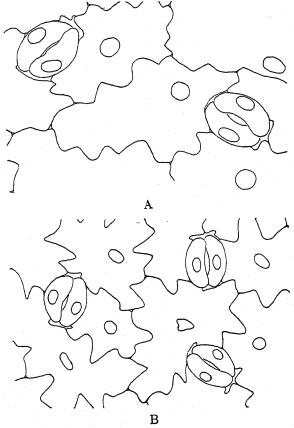


Fig. 2. Stomata of the lower epidermis from mature leaves of tetraploid (A) and diploid (B) alfalfa. ×413.

of an axial row of six cells (Cooper, 1935). Proembryos at this stage are usually present 72 hours after pollination.

The flowers of a number of racemes were tripped, and the heat treatment was applied for a period of one hour at intervals ranging from 36 to 50 hours after tripping. Each inflorescence was treated once, the treatment being at a given constant temperature within the range 41° to 46°C. The apparatus used was designed by Barlow and Brink and has been described in detail by the latter (1936). The same device was used by Atwood (1936) in the production of tetraploid and aneuploid forms of Melilotus alba.

The 400 seeds obtained from the treated racemes were grown in the greenhouse, and the somatic number of chromosomes of each resulting plant was determined by means of aceto-carmine smears. One

plant proved to be tetraploid (fig. 1A, 2n = 64); all the others were diploid (fig. 1B, 2n = 32). The seed from which the tetraploid was obtained came from a raceme which had been subjected to a temperature of  $42^{\circ}$ C. during the interval between 47 and 48 hours after pollination. The writer is not aware of any other case of tetraploidy in alfalfa, either natural or induced, and therefore assumes that the 4n plant obtained can be ascribed to the heat treatment.

Many tetraploids are characterized by giant types of vegetative organs. Such giantism did not occur in the alfalfa tetraploid. There were, however, measurable increases in size. The stems, leaves, and the individual flowers of each raceme were somewhat larger than those of the diploid plants, the greater size of the flowers being most noticeable. A high percentage of the pollen grains from the 4n individual were abortive. The average diameter of the plump pollen grains was distinctly greater, the computed volume of such grains being approximately twice that of grains from 2n plants. The mature leaves of the tetraploid have larger and more widely spaced stomata than do those of the diploid (fig. 2A, B). The stomata of the former average 29.6  $\mu$  in length and 22.2  $\mu$  in width as compared with an average length and width of 22.8  $\mu$  and 16.4  $\mu$ , respectively, in the latter. The epidermal cells are likewise larger in the tetraploid individual. Similar size relations of pollen grains, stomata, and epidermal cells have been reported in artificially induced tetraploid maize (Randolph, 1935).

No viable seeds were obtained from the tetraploid plant. Material for the study of meiosis was collected, and a number of cuttings was made before the plant was transferred to the field. Fifteen of the plants obtained from cuttings were brought into bloom in the greenhouse during April and May, 1938. The flowers were regularly tripped in the hope of obtaining seeds. Twelve pods were formed from which an equal number of more or less shriveled seeds were harvested. Three of these seeds germinated, and the young plants are growing well. The stomata in each of these offspring are comparable in size to those of the parent plant, and the somatic chromosome number in each case is 64.

Chromosome behavior.—Both bivalent and quadrivalent associations of chromosomes appear at diakinesis and on the equatorial plate of the first meiotic division in microspore mother cells. Twenty-four bivalent and four quadrivalent groups are present in the diakinesis configuration shown in figure 1C. A polar view of the first meiotic equatorial plate presents five quadrivalent groups, the other chromosomes being associated as bivalents (fig. 1D). Lateral views of each plate examined showed some quadrivalents (fig. 1E, F), the number ranging from three to six. No trivalents or univalents were observed. A portion of the chromosomes on each equatorial plate usually disjoin in an irregular manner. A number of lagging chromosomes are

present in most anaphase figures of the first meiotic division (fig. 1G, H). In occasional figures (fig. 1H) there is some evidence that both members of a bivalent may pass to the same pole. The lagging chromosomes usually arrive at the poles in time to become incorporated in the daughter nuclei, and rarely during the interphase do chromosomes appear in the cytoplasm (fig. 11). The second meiotic division is likewise characterized by lagging chromosomes which often fail to be included in the spore nuclei (fig. 1J). These laggards may form the nuclei of small abortive microspores, or a small nucleus formed by one or more of these bodies may be included with a larger nucleus in a two-nucleate microspore (fig. 1K). A typical tetrad of four uninucleate spores is seldom seen. In macrosporogenesis the chromosomes behave as in microsporogenesis, and macrospores with extra small nuclei have been observed. Occasionally one and sometimes two of the ovules in an ovary possess embryo sacs that are apparently normal at the open flower stage. Pollen tubes are present in the cavities of ovaries collected 48 hours after tripping, and in two instances stages in fertilization were observed.

#### SUMMARY

Racemes of tripped flowers of *Medicago sativa* were subjected to heat treatment at an early stage in the development of the pro-embryo.

One tetraploid individual (2n = 64) was obtained from a population treated at 42°C. during the interval between 47 and 48 hours after pollination.

The tetraploid plant is similar to diploids in general habit. It possesses somewhat stouter stems and larger leaves and flowers. The stomata, epidermal cells, and pollen grains of the tetraploid are appreciably larger than those of the diploid.

The number of quadrivalents present at diakinesis in the microsporogenesis of the tetraploid plant varies from three to six.

Lagging chromosomes are present on both the heterotypic and homoeotypic spindles.

The induced tetraploid is highly sterile. Three offspring have been obtained from seeds resulting from self-pollination, all of which are tetraploid.

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# LEAF POSITION IN AILANTHUS ALTISSIMA IN RELATION TO THE FIBONACCI SERIES $^{\scriptscriptstyle 1}$

## P. A. Davies

THE WRITER has shown that in Ailanthus altissima Swingle, although there appears to be a normal leaf arrangement, different phyllotaxis systems frequently occur (Davies, 1937). He also stated that the divergent angle of the apparent normal arrangement was approximately 135°, but offered no statistical proof. The present paper is a statistical study of the normal leaf arrangement and its relation to the Fibonacci series.

Schimper (1829, cf. Braun, 1831, p. 197, and Martin, 1833, p. 317), Braun (1831), and Bravais (1837), seeking an explanation for their studies on phyllotaxis, adopted the Fibonacci summation series (0, 1, 1, 2, 3, 5, 8, 13, etc.) and the principles of the "Spirals of Archimedes" to construct the Fibonacci fractional series (1/2, 1/3, 2/5, 3/8, 5/13, etc.) of leaf arrangement.

<sup>1</sup> Received for publication November 2, 1938.

Wiesner (1875) pointed out that under natural conditions the constant divergence expressed by the fractional series does not hold. In any system there is a variation in divergent angles. This postulate is supported by the studies of numerous workers on a great variety of plants.

Not only does the divergent angle fluctuate on any stem, but different fractional series may be present. Weisse (1926) found a change in phyllotaxis just below the floral structures in several species in the family Anonaceae. Schaffner (1926) has observed from studies on Cannabis sativa that frequently there is a change from opposite to alternate phyllotaxis near or in the inflorescence. Skutch (1927), working with Musca sapientum var. hort., observed that the phyllotaxis changed from about 2/5 in very young plants to 3/7 in half-grown plants and finally to 4/9 in the mature plants.

Toichi (1930) found that in Gardenia jasminoides there is a change from the opposite to a secondary whorl, which later may be replaced by the opposite. Bilhuber (1933) shows that in species of the families Cacteae and Euphorbiaceae beside the most often limit-divergence of 137° 30' 28", there also occur limit-divergences of 99° 30′ 43″, 77° 57′ 19″, and 64° 4' 43". Snow and Snow (1933, 1935) were able to alter the normal phyllotaxis ratio through experimental displacement of primordia at the apex in Lupinus albus and the diagonal splitting of the apices of Epilobium hirsutum. Of the 70 pieces of tissue formed by the split in Epilobium hirsutum, 50 regenerated strongly and grew new apices. Thirty-seven of the new apices immediately developed a spiral phyllotaxis, 5 were nearly decussate or becoming so, and 8 developed certain other arrangements. Davies (1936, 1937) has shown for Ailanthus altissima that frequently a new phyllotaxis system is established. The system which appears depends on the position of the first abnormally placed leaves.

A divergent angle has been sought which would express the most ideal divergence. Wiesner (1902) has indicated that the ideal angle is one in which the leaves are arranged in a single spiral sequence with a minimum overlapping. The mathematical expression of this "ideal angle" is 137° 30' 28". As the "ideal angle" is purely mathematical and based upon the Fibonacci fraction series, the angle is known as the "Fibonacci angle." There have been numerous attempts to correlate the divergent angle in actual counts with the theoretical Fibonacci angle. Church (1920) found in Sempervivum calcaratum and in Cobaea scandens, both of which have a 3:5 system, a divergent angle of 137° 38' 50" and 137.65°, respectively. The results of the observations of Schoute (1925) on Peperomia blanda, P. Langsdorfii, P. pulchella, and P. rubella show a divergent angle of about 140°. Salisbury (1931) gives the divergent angles for two winter rosettes of Ranunculus parviflorus. In both rosettes the average divergence was 137.3°. Snow and Snow (1931) found that the mean value of 52 divergent angles measured on Lupinus albus was 136.3°. Bilhuber (1933) found that in a large number of species in the families of Cacteae and Euphorbiaceae the divergent angles approximate the Fibonacci angle, although other divergent angles of lower values were present.

Cook (1914) states that the theories of phyllotaxis based upon leaf position on the mature stem or primordial positions at the developing stem apex do not give an adequate explanation from the botanical viewpoint of how the system originates. Van Iterson (1907) has found that in the seedlings in numerous species of plants the first two leaves arise at approximately 180° from each other or approximately 90° from the cotyledons. The divergent angles of the subsequent leaves do not approach the regular angles expressed by the fractional series until several leaves have arisen. Martinovsky (1930) found in various species of

Gagea that the leaves up to the fifth position have a distichous distribution; above that a secondary spirodistichous spiral appears. Toichi (1930) found in Gardenia jasminoides that both opposite and 3-whorled leaves occur; the opposite is the regular type, giving rise to the secondary whorls. Snow and Snow (1931) observed that the first two leaves in Lupinus albus arise at approximately 180° from each other, and then there is a gradual approach to the angle which can be considered normal.

The studies presented here by the writer are of the normal leaf arrangement in Ailanthus altissima with reference to: (1) position in a phyllotaxis series, (2) variation of the divergent angles, (3) closeness to which the mean divergent angle in actual counts approximates the Fibonacci angle, and

(4) the origin of the normal system.

MATERIALS AND METHODS.—In determining foliar position on the mature shoot, only the long, rapidly growing shoots were used. The shoots selected showed at least three complete foliar cycles. Since the shoots were selected after leaf fall, leaf scars were used as indicators of foliar position. A specially constructed apparatus was employed in measuring the divergent angles. The lower end of the shoot was securely fastened to a base platform and the upper part projected through an opening in the center of an adjustable platform. On the adjustable platform was a circle four feet in diameter divided into one-tenth degrees. The center of the circle was in the center of the opening through which the shoot projected. By moving the platform up or down, the divergent angles between foliar positions and between foliar cycles could be measured. The uppermost leaf scar was the initial point for measuring foliar divergence.

For determining the divergent angles at the developing apex, rapidly growing young shoots about 25 centimeters in length were selected. The shoots were long enough so that adjustment from the opposite to the whorled phyllotaxis had taken place. About two centimeters of the tips were removed, fixed in formalin-acetic-alcohol, as given by Chamberlain (1932), dehydrated by the Zirkle (1930) method, and embedded in paraffin. Serial sections were cut, stained with crystal violet, and mounted in balsam. Camera lucida drawings were made at different levels, so that the angular divergence of the leaf primordia and leaves through complete cycles could be determined. The divergent angles were measured in the camera lucida drawings by means of a protractor. The first definite leaf primordium, and not the primordial bulgings at the apex, was the initial point for the measurements.

Because it was impossible to obtain suitable transverse sections of resting buds, developing buds about 0.6 to 1.3 centimeters in length were used in measuring divergent angles in bud structures. Serial sections were made and the divergence angles measured in the same manner as described for the stem tip. The two outer cataphylls (A and A'; Davies, 1937) appear opposite each other. Cata-

phyll A was used as the initial point in divergent angle measurements.

The divergent angles of the cotyledons and leaves of young seedlings were measured in two ways, each serving as a check on the other. Photographs were made at different stages in seedling development, and the divergence angles as recorded on the photographs were measured with a protractor. Serial sections at different stages of seedling development were made and measured by the method described for the study of the divergence angles at the tips of rapidly growing young shoots. One of the cotyledons was used as the initial point in the divergent angle measurements.

Discussion.—The position of Ailanthus altissima in relation to a phyllotaxis series appears to be a 3/8 or 3:5 arrangement. The mathematical interpretation of the 3/8 arrangement would be that eight leaves compose a cycle. The leaves are distributed in three revolutions about the stem so that each leaf has a divergence of 135° and the first leaf of each cycle is directly superimposed over the first leaf of the preceding cycle. Wiesner (1875) and others have shown that the constant divergence of the fractional series does not hold, and in many cases great variations occur in both foliar and cyclic divergences. Salisbury (1931) has interpreted the 3/8 system that each leaf is not required to have an exact divergent angle of 135° and that the ninth leaf does not have to be directly superimposed over the first leaf, but that three members must invariably occur at each revolution of the stem. In all cases of Ailanthus in which the mean foliar divergence approximates the Fibonacci angle, three leaves occur in each revolution and eight leaves are present in three revolutions of the stem. The average divergence of leaf positions on mature shoots was 136.11°, or 1.11° above that of the 135° distribution of the 3/8 fractional series. The mean divergence of 685 angles measured at different areas on the plant (mature shoots, shoot tips, developing buds, and young seedlings) was 137.666° or only 2.666° greater than the constant angle expressed by the 3/8 arrangement. With a standard deviation of 8.583° from the mean divergence in the 685 angles measured, the 2.666° deviation from the 135° of the

Table 1. Angular divergence of three consecutive cycles on long shoots. First cycle indicates the divergent angle between the first leaf position (1) of the first cycle and the first leaf position (9) of the second cycle; the second cycle, between the first leaf position of the second cycle (9) and the first leaf position of the third cycle (17); etc. All measurements are in degrees.

		Foliar cycles	
Shoot number	First cycle 1-9	Second cycle 9–17	Third cycle 17-25
1	14.3	3.7	8.3
2	0.5	9.0	3.9
3	8.1	8.2	16.0

3/8 fractional series does not appear to be so large. Cyclic divergent variations in three consecutive cycles on three shoots are shown in table 1. These were selected at random from measurements on 12 shoots. In shoot number 1, the first cyclic divergence was 14.3°; the second, 3.7°; and the third, 8.3°. Shoots numbered 2 and 3 show similar large cyclic divergent variations. In the 36 cycles measured, the divergent range was from 0.5° to 30.3°, with an average of 10.31°. In none of the cycles

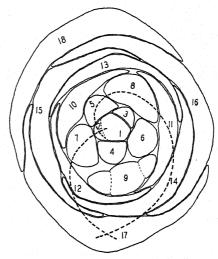


Fig. 1. Camera lucida drawing of a transverse section of a developing bud of *Ailanthus altissima*. Eighteen cataphylls, leaves, or leaf primordia are shown. Dotted lines are drawn through the approximate centers of units 2-5-8-11-14-17 and 2-7-12-17, respectively.

was the first leaf in each cycle directly superimposed over the first leaf of the preceding cycle, although in several cases the divergence was not great.

Church (1920, fig. XII) shows a drawing of a transverse section of the apex of Sempervivum calaratum. On the drawing appears a curve-tracing with a line passing through units 2-5-8-11-14-17-12-7-2. This he interprets to be a 3:5 phyllotaxis construction. Figure 1 shows a camera lucida drawing through the apex of a developing bud of Ailanthus altissima. The curve-tracing obtained by drawing a line through units 2-5-8-11-14-17-12-7-2 has the same logarithmic expression as Church has given for Sempervivum calcaratum and which he expresses as a 3:5 system. He also found in Sempervivum calcaratum and in Cobeae scandens, both of which have a 3:5 system, a divergent angle of 137° 38′ 50" and 137.65°, respectively. The mean divergence of the 685 angles in Ailanthus altissima after a normal phyllotaxis was established was 137.666° or 137° 39' 57". This very closely approximates the divergent angle in Sempervivum calcaratum and Cobeae scandens.

The data expressing the variations of the divergent angles and the relation of the cyclic averages of the divergent angles in normal distribution are presented in four groups of measurements: (1) leaf

TABLE 2. Divergent angles of foliar distribution and the averages of the divergent angles of foliar distribution covering two cycles on three long mature shoots. All measurements are in degrees. divergent angle between leaves one and two; leaf order 2-3, between leaves two and three; etc. Leaf order 1-2 is the

								Leat order	rder								
Shoot number	1-2	1-2 2-3 3-4	3-4	4-5	2-6	2-9	7-8	6–8	9-10	10-11	11-13	12-13	13–14	14-15	15–16	16-17	Ave. divergence
	138.5	132.5	143.2	136.2	127.5	137.1	137.0	138.0	125.4	136,4	137.2	132.9	133.9	138.2	134.5	135.5	135.25
G	129.0	141.1	135.1	140.3	140.3	125.3	141.1	136.6	136.2	143.5	134.5	140.1	133,4	131.2	138.3	136.0	135.75
<b>.</b>	127.3	142.6	140.1	143.0	127.3	140.1	148.4	134.9	150.0	142.2	132.3	125.1	132.2	136.1	143.0	137.1	137.56

positions on the mature stem, (2) primordial and leaf positions at the tip of rapidly growing long shoots, (3) leaf positions in developing buds, and (4) leaf positions on young seedlings.

Table 2 shows the divergent angles of foliar distribution and the averages of the angles of foliar distribution in two complete cycles on long mature shoots. The data were selected from the measurements of three complete cycles on 9 shoots. selections were made so that the first and last divergent angles approximated as nearly as possible the average for the two cycles and at the same time show both an angular variation within the cycle and a difference in the average variation in the two cycles on different shoots. The variations in the 216 angles measured was from a minimum of 124.7° to a maximum of 150.1°, or a range of 25.4°. The variation of the averages of the angles in the three cycles on the different shoots was from a minimum of 135.1° to a maximum of 140.3°, or a range of 5.2°. The average of the divergent angles for all measurements on the mature shoot was 136.11°.

The angles of foliar divergence and the averages of the angles for foliar distribution in a single cycle on each of four shoot tips selected from the 20 studied are shown in table 3. The selections were made so that the last divergent angle (8-9) approximated the average for the divergent angle in the cycle and at the same time showed both an angular variation within the cycle and a difference in the average variations between the cycles. The average variation in the 160 angles measured was from a minimum of 118.3°, to a maximum of 151.2°, or a range of 32.9°. The variation of the averages of the angles in the cycles was from a minimum 134.75° to a maximum of 141.70°, or a range of 6.95°. The average for all divergent angles measured was 137.51°.

The angle of foliar divergence and the averages of the angles of foliar divergences in complete cycles at the shoot tip of four developing buds after the average divergent angle approximated the Fibonacci angle is shown in table 4. The selections were made so as to show the variation of the divergent angle within the cycles and the average of the divergent angle in complete cycles. The angular variations in the 160 angles measured was from a minimum of 114.6° to a maximum of 157.5°, or a range of 42.9°. The variation of the averages of the angles in the cycles was from a minimum of 136.11° to a maximum of 141.65°, or a range of 5.54°. average for all divergent angles measured was 138.20°.

Table 5 shows the angular divergence and the averages of the angles of foliar divergence in the first complete cycles on four young seedlings after a normal phyllotaxis system was established. The data of the four seedlings shown in the table were selected from data on ten seedlings studied to show the variation of the divergent angles within the cycles and also a variation in cyclic averages. The angular variations in the 80 angles measured was from a minimum of 118.3° to a maximum of 165.4°,

Table 3. Divergent angles of foliar distribution and the averages of the divergent angles of foliar distribution in the first foliar cycle at the stem tip in four rapidly growing young shoots. Leaf order 1-2 is the divergent angle between leaf primordia one and two; leaf order 2-3, between primordia two and three; etc. All measurements are in degrees.

				Leaf	order				
Shoot number	1-2	2–3	3-4	4-5	5–6	6-7	7-8	8-9	Ave, divergence
1	124.3	146.1	130.8	135.5	134.2	142.0	130.9	134.7	134.81
2	130.1	138.2	133.8	139.2	135.7	136.4	135.0	134.1	135.31
3	135.8	139.8	132.7	137.1	142.1	136.9	139.9	142.1	138.30
4	139.8	143.5	139.7	141.5	132.5	147.0	134.3	146.6	140.61

Table 4. Divergent angles of foliar distribution and the averages of the divergent angles of foliar distribution in complete cycles at the shoot tip in four developing buds after the average divergent angle approximated the Fibonacci angle. Leaf order 1-2 is the divergent angle between leaves one and two; leaf order 2-3, between leaves two and three; etc. All measurements are in degrees.

					Leaf	rder				
	Shoot number	1–2	2-3	3-4	4-5	5-6	6-7	7–8	8-9	Ave. divergence
-	1	135.3	138.4	136.5	138.2	142.0	126.0	142.2	136.7	136.91
	2	138.6	126.2	157.5	122.2	143.1	140.4	134.5	139.2	137.71
	3	136.7	133.3	145.3	132.6	144.4	130.0	147.1	137.5	138.36
	4	139.0	152.4	128.6	144.2	126.2	155.0	137.5	134.3	139.65

Table 5. Divergent angles of foliar distribution and the averages of the divergent angles of foliar distribution in complete cycles on four young seedlings after a normal distribution was established. Leaf order 1-2 is the divergent angle between leaves one and two; leaf order 2-3, between leaves two and three; etc. All measurements are in degrees.

				Leaf	order				
Seedling number	1–2	2–3	3-4	4-5	5-6	6-7	7-8	8-9	Ave. divergence
1	134.2	139.7	131.9	137.6	127.0	145.4	132.6	140.7	136.13
2	137.6	141.3	132.4	144.6	134.9	140.0	133.5	138.7	137.93
3	139.2	147.6	130.3	143.4	135.3	145.0	133.6	140.1	139.31
4	133.9	140.3	135.3	151.9	135.3	151.1	137.3	146.0	141.40

Table 6. Divergent angles of cataphyll distribution in the first nine units to form in four developing buds. A and A' are the outer cataphylls and the first two to develop. B the third cataphyll follows A and A' in development, B' follows B; etc. All measurements are in degrees.

				Cata	aphyll or	ler			
Bud number	A-A'	Λ'-Α	A-B	B-B'	B'-1	1-2	2-3	3–4	4-5
1	180.0	180.0	88.7	156.3	80.7	150.2	124.6	157,5	139.0
2	178.1	178.1	90.0	156.0	88.7	145.3	125,2	138.2	127.5
3	178.7	178.7	87.9	179.0	61.3	125.6	137.3	142.6	135.3
4	179.8	179.8	91.1	154.7	88.5	148.8	139.0	126.5	146.6

or a range of 47.1°. The variation of the averages of the angles in the cycles was from a minimum of 136.13° to a maximum of 141.40°, or a range of 5.27°.

Tables 6 and 7 show the variations in the divergent angles in bud and seedling development before a normal phyllotaxis system is established. As the tables indicate, variations are usually greater before

Table 7. Divergent angles of cotyledon and foliar distribution in the first twelve units to form in three developing seedlings. C and C' are the two cotyledons. Units 1, 2, 3, 4, etc., are successive leaves. C-1 is the divergent angle between a cotyledon and the first leaf; C-2, between the same cotyledon and the second leaf; 2-3, between the second and third leaves; etc. All measurements are in degrees.

					Cot	yledon	and lea	f order	44.			
Seedling number	C-C'	C'-C	C-1	C-2	2–3	3-4	4-5	5-6	6-7	7-8	8-9	9–10
1	179.1	179.1	88.7	88.4	90.7	163.2	121.4	145.5	133.9	140.4	135.3	143.9
2	180.0	180.0	90.4	89.6	88.9	167.3	113.4	154.7	127.7	146.3	133.3	139.2
3	178.2	178.2	88.3	91.3	90.3	177.3	107.6	165.2	122.0	149.3	131.7	143.2

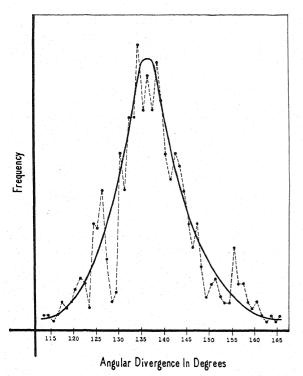


Fig. 2. Distribution curve of the divergent angle frequencies for 685 angles. The broken lines, which connect the frequency points, indicate the variation of the frequencies. The solid black line is a smoothing out of the frequency curve.

a normal phyllotaxis has been established than after one has been established.

Figure 2 shows a frequence distribution curve for 685 divergent angles from different areas of the plant (mature shoot, rapidly growing shoot tips, developing buds, and young seedlings). A broken line connects the frequence points and shows clearly wide frequency variation. The solid black line is a smoothing out of the frequency curve and shows a rather uniform distribution curve. The variations in divergent angles were from a minimum of 113.3° to a maximum of 165.4°, or a range of 52.1°.

It is clearly evident from the data presented that wide variations of divergent angles are present on the same shoot and on different shoots, even after the normal phyllotaxis system is established. This is in agreement with the postulate of Wiesner (1875) and others that a constant divergence as expressed by the fractional series is not maintained under natural conditions.

The closeness to which the mean angular divergence approximates the Fibonacci angle is given from calculations on data presented in figure 2. The mean divergence of the 685 angles measured after a normal phyllotaxis system was established is 137.666° or 137° 39′ 57″. Comparing this with the Fibonacci angle (137° 30′ 28″), a difference of only 0° 9′ 29″ or .159° is evident. The standard deviation from the mean is 8.583°. It is interesting to note that with such a wide deviation the mean divergent angle should so closely approximate the Fibonacci angle. It is clearly the result of the particular group of angles measured, for any other group of measurements, even with a greater number of angles, may give a much greater mean divergence.

Cook (1914) states that the theories of phyllotaxis based upon leaf position on the mature stem or primordial positions at the developing apex do not give an adequate explanation from the botanical viewpoint of how the system originates. Van Iterson (1907) for numerous plants, Martinovsky (1930) for Gagea, Toichi (1930) for Gardenia jasminoides, and Snow and Snow (1931) for Lupinus albus, show that the first leaves to appear have a decussate distribution and that gradually there is an approach to the angle which may be considered normal. The normal phyllotaxis system in Ailanthus altissima as expressed on the mature shoot or the developing stem apex (tables 2 and 3) has its origin in either bud or seedling development.

Table 6 shows the origin of the normal system and the angular divergence for the first nine cataphylls to appear in bud development. The four examples were selected from 15 buds studied in order to show the variation in divergent angles and the level at which a normal distribution was established. The outer cataphylls (A and A') develop opposite each other or at an angle of approximately 180°. B, the third cataphyll to develop, appears basically and approximately equally distant (90°) between A and A'. B', the fourth cataphyll to appear, develops either in a clockwise or counter-

clockwise direction from B.2 Its divergent angle (shown in the table by B-B') in the four examples shows a variation from 154.7° to 179°. The average for all 15 buds was 162.77°. The divergent angle of unit 5 (B'-1) was the smallest angle in the bud. It was the smallest consistent angle of all divergent angles measured. The average for the 15 buds was 77.4°. Above the fifth unit, the divergent angles tend to gradually approach an angle comparable to the Fibonacci angle. The normal distribution, approximating the Fibonacci angle, was not established at the same level in all buds. In bud number 1 the first angle to approach the Fibonacci angle was unit 9 (4-5); in number 2, unit 8 (3-4); and in numbers 3 and 4, unit 7 (2-3). In the 15 buds studied, four approached the Fibonacci angle in the 7th units, 7 in the 8th unit, and 4 in the 9th unit.

The cotyledons and foliar distributions in the first twelve units to form in three developing seedlings and the establishment of a normal phyllotaxis are shown in table 7. C and C' are the two cotyledons, and they appear opposite or at an angle of approximately 180°. The first two leaves to develop appear opposite and approximately 90° from the cotyledons. The third leaf (2-3) is superimposed over one of the cotyledons (cotyledon C) or approximately 90° from leaf 1 or 2. The fourth leaf appears in an angle of least influence between either leaf 1 and cotyledon C or leaf 2 and cotyledon C. The average for the divergent angle for leaf number 4 in the three shoots shown in the table is 169.26°. Above the fourth leaf there is a gradual approach toward an angle which may be considered normal. In seedling number 1 this angle was established by the seventh leaf, while in 2 and 3 by the ninth leaf. In the 20 seedlings studied, two devel-

<sup>2</sup> A transverse drawing of a bud and the designation of the symbols A, A', B, and B' are given in a previous paper (Davies, 1937).

oped the normal arrangement with the seventh leaf; six with the eighth leaf; seven with the ninth leaf; four with the tenth leaf; and one with the eleventh leaf

The data show that the development of the normal leaf arrangement in Ailanthus is gradual. Beginning with the one-half or decussate distribution there is a gradual approach to the normal, which approximates the 3/8 distribution. This gradual approach from the decussate to the normal arrangement is in agreement with the results of Van Iterson, Martinovsky, Toichi, and Snow and Snow.

#### SUMMARY

The position of Ailanthus altissima in a phyllotaxis series appears to be a 3/8 or 3:5 arrangement—that is, the mean foliar divergent angle approximates the 135° distribution of the fractional series.

Large variations in divergent angles occur even on the same shoot. The variation of angular divergence in 685 measurements on mature shoots, rapidly growing shoot tips, developing buds, and young seedlings was from a minimum of 113.3° to a maximum of 165.4°, or a range of 52.1°.

The mean angular divergence of 685 foliar positions was 137.666° or 137° 39′ 57″. This very closely approximates the Fibonacci or "ideal angle" of 137° 30′ 28″. The standard deviation from the mean was 8.583°.

The normal phyllotaxis system has its origin in the one-half or decussate arrangement in bud or seedling development. The normal system was established in bud development between the seventh and ninth units and in seedling development between the seventh and eleventh units.

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## A COMPARISON OF TWO QUANTITATIVE AVENA TECHNIQUES IN THE DETERMINATION OF 3-INDOLE ACETIC ACID <sup>1</sup>

Richard H. Goodwin

A BIOLOGICAL method for the quantitative determination of minute amounts of auxin was described by Went (1928), who employed as a test object the coleoptile of Avena sativa. Although other species of plants have since been used with some success, none of them has obtained such universal popularity. Went's method employs seedlings grown in water cultures at a high (85-90 per cent) relative humidity. Boysen Jensen (1935, 1937), however, has described a somewhat different Avena technique—a further development of the method used by Nielsen (1924)—, in which the plants are cultivated in soil at a much lower (30-45 per cent) relative humidity. He points out that the plants, although somewhat more variable, are also more sensitive when grown under these conditions. Since these two methods of auxin assay have both been used up to the present time, it was thought worth while to carry out a series of experiments comparing their relative advantages.

In the following investigation the activity of several concentrations of 3-indole acetic acid was tested from time to time over a period of several months both by the method of Went and by that of Boysen Jensen. A fresh standard solution was made up each time by dissolving 5 mg. of 3-indole acetic acid (LaRoche) in 5 cc. of peroxide-free ether and then adding 500 cc. of distilled water. Of this standard solution 1 cc. was diluted with distilled water to twice the concentration to be tested, and 5 cc. of this solution were added to an equal volume of 3 per cent agar at 60°C. Thimann and Schneider (1938) have demonstrated that direct mixing of 3-indole acetic acid solutions with melted agar is preferable to soaking agar blocks in the solution. Of the agar mixture 2.5 cc. were poured out on a 5 × 5 cm. glass plate, and as soon as the agar had hardened, the blocks  $(2 \times 2 \times 1)$ mm.) to be used in the tests were cut out with parallel knives. Although blocks of 4 mm.3 are smaller than those used by many workers, difficulty

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This investigation was carried out in the Plant Physiology Laboratory of the University of Copenhagen during the tenure of an American-Scandinavian Foundation Fellowship. The writer is deeply grateful to Professor P. Boysen Jensen for making available the facilities of his laboratory and for his unfailing interest in the work.

due to drying out of the blocks was not experienced.

Victory oats, a Swedish variety generally used in phytohormone research, served as the test object. In Copenhagen Victory oats have recently been substituted for the variety Gul Naesgaard. In comparing the soil-culture technique of Boysen Jensen (1935) with the water-culture technique of Went (1928), two series of tests were carried out. For the sake of brevity, the cultural conditions and technical details of the two methods as followed in this investigation are outlined in table 1. The dark room was maintained at  $23 \pm 1^{\circ}\mathrm{C}$ ., a temperature intermediate between that used by Went and that used by Boysen Jensen.

The magnitude of each curvature was determined in two ways: (1) the angle between the tip of the coleoptile stump and the uncurved base was measured with a protractor from shadow prints which were made on bromide paper at the end of each test (method of Went), and (2) by matching the concave surface of the curved portion of the coleoptile with the corresponding arc in a graded series (radii of curvature ranging from 6 to 80 mm.; lengths marked off in mm.) drawn on a scale (Boysen Jensen, 1935) l, the length of the curved portion of the coleoptile, and r, the radius of curvature, were measured directly. The angle of curvature,  $\psi$ , was calculated from the formula,  $\psi = 360 l/2 \pi r$ . In Copenhagen curvatures are measured by d, the difference in length between the convex and concave sides of the coleoptile, which can be calculated from the formula, d = tl/r (Purdy, 1921), where t, the thickness of the coleoptile, is taken as a constant. By substituting tl/r for d in the formula d = $\psi$  2  $\pi$  t/360 (Boysen Jensen, 1935), the formula,  $\psi = 360 \ l/2 \ \pi \ r$  can be derived.

In forty tests the mean difference between the larger and smaller curvatures obtained by each method was  $1.01^{\circ} \pm 0.19^{\circ}$ . This is not a systematic error, since the values for the measured angles averaged only  $0.16^{\circ} \pm 0.19^{\circ 2}$  less than those for the angles calculated by the second method. Although Dolk (1930) has shown that the curvatures of Avena coleoptiles are not true arcs of circles and, hence, that the measured value of r can be only an approximation, any error thus introduced

<sup>2</sup> The standard deviation of the mean is given.

is rather insignificant in the determination of large angles. In the case of small angles, on the other hand, direct measurement from shadow prints is more accurate, especially since values of r greater than 80 mm. are neglected. Furthermore, shadow prints afford permanent photographic records not only of the magnitudes but also of the shapes of the curvatures.

The curvatures obtained by each method (outlined in table 1) for five concentrations of 3-indole acetic acid and the dates of the tests are given in table 2. Each value is the mean of 12 plants. It may be seen that, for a given concentration, curvatures obtained with Boysen Jensen's technique were nearly twice as large as those obtained with Went's method. There are probably at least three factors partially responsible for this difference: (1) greater sensitivity of the soil-grown plants, (2) a 35- to 40- minute difference in interval between decapitation and application of the agar block (or IDP, see Schneider and Went, 1938), and (3) a 70-minute difference in photographing time. The standard deviations of the averages in table 2, due both to individual variations in the plants and to slight changes in conditions from day to day, are of nearly the same magnitude with each method. Hence, the coefficient of variability of the averages is considerably smaller for tests with Boysen Jensen's technique. Juel (1936) obtained comparable variation in tests with this method in the same laboratory. It should be pointed out, however, that the standard deviations of individual tests with Went's method are larger than those obtained by Went (1928), van der Weij (1931), et al. With more careful control of the temperature and humidity, the variability of both methods might well have been reduced.

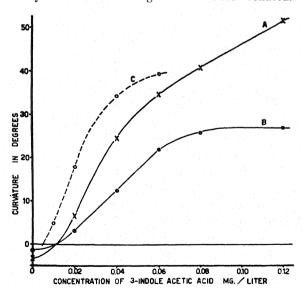


Fig. 1. Curvature of Avena coleoptiles for various concentrations of 3-indole acetic acid. A, soil-culture technique of Boysen Jensen (1935). B, water-culture technique of Went (1928). C, deseeded, water-culture technique of Skoog (1937).

TABLE 1.

Soil-culture technique of Boysen Jensen (1935) Water-culture technique of Went (1928)

Large seeds selected; husks not removed.

Grains soaked for 24 hours in 5 mm. of water at 18°C.; exposed to daylight.

Large seeds selected and dehusked.

Seeds soaked for 3 hours; then laid out, groove down, on wet filter paper in petri dishes for 24 hours at 22-24°C.; exposed to some red light.

Seeds planted in glass holders with the roots in tap water (pH about 7.4).

Grains planted vertically in glass vials ( $20 \times 100$  mm.) filled very loosely with sifted, sandy loam (water content 26-28 per cent (Juel, 1936)), the apex just above the surface of the soil. The soil may be used repeatedly for about a month if kept in a covered container and moistened after each test.

Plants grown in a dark room at 22-24°C. (Boysen Jensen (1935) used a temperature of 21-21.5°C.) under containers for the first 48 hours and in partially-covered boxes at 35-45 per cent relative humidity for the last 24-28 hours.

Tests carried out 72-76 hours after planting, when the coleoptile tips were 18-20 mm, above the soil surface.

3 mm. of the coleoptile decapitated by nicking the  $b \ ro \ a \ d$ , slightly-concave side with a razor and breaking off the tip. The stump was moistened with a drop of water.

Agar block 4 mm.<sup>3</sup> placed on the broad, concave side within 5 minutes of decapitation. Plants placed under 2-liter bell jars lined with wet filter paper during the test. (Tests with the block placed on the narrow side proved to be more variable.)

Curvatures measured 180 minutes after application of the agar blocks.

Plants grown in a dark room at 22–24°C. (Went, 1928, and subsequent workers have used 25°C.) in a moist chamber with a relative humidity of about 85 per cent.

Tests carried out 48-52 hours after planting when the coleoptiles had reached a length of 20-30 mm.

4-5 mm. of the coleoptile decapitated by nicking a narrow side and breaking off the tip. The primary leaf pulled loose. Excess water removed from the stump with filter paper.

Agar block 4 mm.<sup>3</sup> placed on the narrow, cut side 40 minutes after decapitation. Plants kept in a moist chamber at about 85 per cent relative humidity throughout the test.

Curvatures measured 110 minutes after application of the agar blocks.

The average curvatures in table 2 are plotted against concentration of 3-indole acetic acid in figure 1. The relation between curvature and concentration for Went's method (fig. 1B) was a linear

TABLE 2.

3-indole acetic	of Boyse	lture technique en Jensen (1935)		lture technique ent (1928)		d water-culture of Skoog (1937)
acid, $\gamma^a$ per liter of 1.5 per cent agar	Date	Curvature in degrees (calculated) ± standard deviation	Date	Curvature in degrees (measured) ± standard deviation	Date	Curvature in degrees (measured) ± standard deviation
	Day Mo.	of the mean	Day Mo.	of the mean	Day Mo.	of the mean
0	1 10 11 10 17 1	$5.4 \pm 1.2$ $3.1 \pm 1.7$ $2.0 \pm 1.0$	1 10 11 10 11 2	$1.3 \pm 0.9$ $1.5 \pm 1.7$ $0.1 \pm 0.8$		
***	17 1 17 1	$2.0 \pm 0.3$ $4.4 \pm 1.4$	23 2 23 2	$1.0 \pm 1.4$ $2.4 \pm 1.4$		
	Average	$3.4 \pm 0.7$	Average	$1.3 \pm 0.4$		
10					23 4 1 5 1 5	$\begin{array}{l} - & 4.7 \pm 1.2 \\ - & 3.5 \pm 0.9 \\ - & 6.6 \pm 1.8 \end{array}$
					Average	$-4.9 \pm 1.6$
20	24 9 1 10 7 10 8 10 8 10	$-10.4 \pm 1.3$ $-5.1 \pm 1.0$ $-4.0 \pm 1.0$ $-5.7 \pm 2.4$ $-6.1 \pm 2.0$	1 10 7 10 8 10 13 10 13 10	$\begin{array}{l} -1.4 \pm 0.5 \\ -2.6 \pm 1.1 \\ -3.5 \pm 0.8 \\ -3.4 \pm 0.8 \\ -4.0 \pm 1.0 \end{array}$	23 4 1 5 1 5	$-15.3 \pm 1.5$ $-18.8 \pm 2.1$ $-19.7 \pm 2.3$
	Average		Average	$-3.2 \pm 0.5$	Average	$-17.9 \pm 2.3$
					11.01.00	11.0 _ 2.0
40	23 9 24 9 28 9 30 9 4 10	$-23.2 \pm 2.4$ $-21.9 \pm 1.3$ $-22.9 \pm 2.0$ $-27.6 \pm 2.7$ $-27.3 \pm 2.4$	30 9 30 9 4 10 4 10 11 10	$-14.5 \pm 1.2$ $-12.3 \pm 1.3$ $-9.2 \pm 1.2$ $-14.6 \pm 1.0$ $-12.1 \pm 1.8$	1 5 1 5 6 5	$-38.7 \pm 3.2$ $-33.1 \pm 2.9$ $-31.4 \pm 2.4$
	Average		Average		Average	$-34.4 \pm 3.8^{b}$
60	4 11 16 11 16 11 16 11 18 11	$-33.4 \pm 2.0$ $-35.4 \pm 1.3$ $-37.4 \pm 1.7$ $-35.0 \pm 1.7$ $-33.0 \pm 1.7$	17 11 26 11 30 11 30 11 23 2	$\begin{array}{c} -24.8 \pm 1.0 \\ -19.7 \pm 1.7 \\ -22.8 \pm 1.5 \\ -24.8 \pm 1.5 \\ -18.3 \pm 0.7 \end{array}$	1 5 1 5 6 5	$-40.3 \pm 2.4$ $-39.2 \pm 4.4$ $-38.9 \pm 4.2$
	Average	$-34.8 \pm 0.8$	Average	$-22.1 \pm 1.3^{\text{b}}$	Average	$-39.5 \pm 0.7$
80	22 9 28 9 4 11 15 11	$-39.8 \pm 2.7$ $-40.8 \pm 2.0$ $-38.4 \pm 2.7$ $-41.8 \pm 2.0$	27 9 7 10 11 10 11 10	$-26.0 \pm 1.6$ $-22.6 \pm 2.0$ $-28.0 \pm 1.2$ $-25.8 \pm 1.2$		
	22 11	$-43.8 \pm 1.7$	23 2	$-27.8 \pm 1.7$		
	Average	$= -40.9 \pm 0.9$	Average	$= -26.0 \pm 1.0$		
120	18 11 19 11 19 11 19 11 22 11	$-56.0 \pm 3.2$ $-49.2 \pm 3.1$ $-51.2 \pm 4.7$ $-46.8 \pm 2.4$ $-56.0 \pm 3.9$	11 2 11 2 16 2 19 2 19 2	$-25.1 \pm 1.8$ $-26.3 \pm 1.4$ $-27.8 \pm 1.9$ $-27.9 \pm 1.0$ $-29.3 \pm 1.6$		
	Average	$= -51.8 \pm 1.8$	Average	$= -27.3 \pm 0.7$		

one with concentrations from 0.01 to 0.06 mg. of 3-indole acetic acid per liter, but strict proportionality was not obtained. This suggests that such proportionality should never be assumed under a new set of conditions until it has been experimentally demonstrated. Thimann and Schneider (1938) point out that the shape of the curvature-

concentration curve for 3-indole acetic acid may be greatly modified by alterations in the number and timing of the decapitations and the concentration of the agar. If proportionality between curvature and concentration does not hold, the amount of active substance in an unknown solution may still be estimated from a curvature-concentration curve.

 $<sup>^{\</sup>rm a}$  1  $\gamma$  = 0.001 mg.  $^{\rm b}$  Approximately the maximum angle.

As suitable absolute units, van Overbeek (1938) suggests 3-indole acetic acid equivalents per liter.

With Boysen Jensen's method the curvature-concentration relationship followed a smooth curve between 0.01 and 0.12 mg. per liter (fig. 1A). A calculation must be made in order to express the amount of active substance in an unknown solution in arbitrary units. Boysen Jensen (1937) uses "WAE," the amount of active substance dissolved in 100 cc. of 1.5 per cent agar which, when applied unilaterally to Avena coleoptiles in 4 mm. blocks at a temperature of 21–22°C., will produce in three hours a curvature with a d value of 1 mm. (or a curvature of approximately 38 degrees). Quantitative determinations can be made over a greater range of concentrations with this method under the conditions described.

With both Went's and Boysen Jensen's methods there appears to be a threshold concentration at about 0.01 mg. per liter, below which no activity can be detected. Among various recent modifications of Went's original technique the deseeded method of Skoog (1937) may be used as a more sensitive test for very low concentrations. Curvatures obtained five hours after application of the agar blocks for water-cultured plants deseeded 18 hours before the tests are given in table 2 and plotted in figure 1C. Under these conditions the threshold concentration is about 0.005 mg. per liter. Skoog (1937) reports still lower threshold values. The deseeded test has been used at Utrecht with great success by Koningsberger and Verkaaik (1938), who showed that the method yields highly reproducible results.

The speed with which tests can be carried out is an important practical consideration. Perfect test plants can be obtained either from soil or from water cultures, but crops of water-grown plants exhibit greater uniformity. For this reason it is not necessary to discard so many water-grown plants before the tests and hence there is an economy of time. The total time required for all operations as outlined in table 1 averages approximately 35 minutes per test of twelve plants for Boysen Jensen's method and about 30 minutes per test for Went's method. If deseeded plants are desired, about 35 minutes per test are required. Permanent records of the curvatures in the form of shadow prints may be obtained by either method. Soilgrown plants are easily handled for photographing by arranging the vials in racks of twelve; but measuring them in this way will further increase the time per test. Calculating the angle from measurements of l and r has the advantage that computation of the results (greatly expedited by means of a table giving angles for values of l and r) can be obtained within five minutes of the end of the test.

Water cultures cannot be conveniently handled in considerable numbers except in a constant-temperature, air-conditioned dark room with a high relative humidity. Where such facilities are not available, the soil-culture method offers a ready means of making auxin determinations. There are two objections to the use of soil, however: (1) the impossibility of reproducing exactly the same conditions in different laboratories, and (2) the difficulty in mantaining the proper moisture-content of the soil. Water cultures obviate these difficulties and lend themselves more easily to experimental modifications such as removal of the primary leaf, deseeding, and alterations of the culture medium. In actual practice it is frequently impossible to compare critically the results obtained in different laboratories with water-cultured test plants, since refinements in the technique are constantly being proposed, no one of which has yet been universally accepted (Avery, Burkholder, and Creighton, 1937; Skoog, 1937; Went and Thimann, 1937; Schneider and Went, 1938; et al.).

## SUMMARY

A comparison of the accuracy and utility of the Avena test in the quantitative determination of 3-indole acetic acid as carried out by Went with water-cultured plants and by Boysen Jensen with soil-cultured plants has been made. Each method offers certain advantages. In a laboratory with no facilities for precisely controlling the humidity of the constant-temperature dark room, Boysen Jensen's test with soil-grown plants may be most simply, accurately, and expeditiously used. In a completely air-conditioned dark room, however, Went's test with water-cultured plants can be more speedily executed and more readily standardized and modified. The relative accuracy of the two methods under such conditions has not been investigated.

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## SOME OBSERVATIONS ON SEXUAL DIMORPHISM IN CAREX PICTA 1

## J. Louis Martens

It is desirable to clarify the problematic relationship of the grasses and sedges. In order to do that, it is appropriate that more sedges be investigated, since heretofore the members of the Gramineae have been more popular subjects for study than have the Cyperaceae. Because of their vital importance, grasses have been studied extensively from both the practical viewpoint and from the standpoint of pure science. The sedges, however, are of incomparable economic importance, and much remains to be known about them before they will be understood as well as the grasses. In an effort to supplement the studies of the Cyperaceae, Carex picta Steudel was chosen for investigation.



Fig. 1. A characteristic plant of Carex picta, Monroe County, Indiana.

<sup>1</sup> Received for publication September 29, 1938. I wish to acknowledge appreciation to Prof. Paul Weatherwax, at whose suggestion this problem was selected, and to Dr. D. M. Mottier under whose direction a part of the work was done. I am also grateful for advice and courtesies extended by other members of the department. Carex picta is a favorable sedge for study. It is typical of the genus Carex in many ways, but it is dioecious. The latter characteristic, however, lends itself to the study of sexual expression in the sporophyte, a subject of general interest. Then this species is sufficiently abundant near Bloomington to have facilitated many collections and field observations.

This study, which was begun in the spring of 1933, includes observations on several different aspects of the plant. Analyses of the flowering habit bring out the stability of its dioecious character. Ontogenetic studies on the floral units portray the relationship of the floret components of each sex and throw light on the comparative degrees of specialization in the staminate and pistillate spikelets. The origin and development of the micro- and megagametophytes, as compared with other species of Carex, complete the observations.

Habit and inflorescences.—This Carex is a tricarpellate species limited to local distribution in the eastern part of the United States. It should not be confused with either of two bicarpellate Carices (Kükenthal, 1909), one native to New Zealand, the other to Asia, to which its Latin binomial has been applied by some authors. It is a common sedge in southern Indiana in the residual soil of the Borden, a sandy shale formation of lower Mississippian age.

C. picta grows as a grass-like plant. It is a vernal perennial species and appears in characteristic tufts (fig. 1) by which it may be recognized in all seasons. These tufts may vary in diameter from a few inches to more than two feet, the male and female plants being practically indistinguishable without inflorescences. During anthesis the male plants produce dark-colored, clavate spikes, each on a filiform scape (fig. 2). The female inflorescences are similar but more slender and of a uniform diameter (fig. 3).

The vegetative and fertile shoots of both sexes grow from an underground stem. They are arranged in a circle, the center of which appears dead. This "fairy ring" effect suggests a radiating rhizome



Fig. 2-3. Inflorescences.—Fig. 2. Male.—Fig. 3. Female.

system physiologically comparable with the radiating growths of mycelium responsible for the "fairy rings" of mushrooms, but it is really brought about in a very different way. The culms grow from the tips of underground branches, but the rhizomes tend to branch often and dichotomously, and the result is that secondary branches fill in an approximately 90' angle (fig. 4, a) formed by the first two primary branches. Secondary branches exterior to this angle tend to complete the circle. Although the system appears nearly perfect above ground, it often develops from a roughly subcircular rhizome system (fig. 5).

This combination of the dichotomously branching rhizome system and its culms is conveniently referred to as a "clump." Inasmuch as a plant must develop under suitable conditions to form a characteristic clump, and it requires several years for one to grow into a ring, all individuals do not appear exactly characteristic. Most of the clumps are distinctly segregated growths. However, they may grow adjoining, and at times the rhizome systems of two separate plants grow interwoven so as to form one clump. When plants of opposite sex are found making up one clump, it is aptly spoken of as a "mixed clump." It should not be forgotten, nevertheless, that in speaking of a mixed clump two plants are involved. This has been verified repeatedly by digging up clumps of this nature and separating the male and female rhizome systems.

The general floral expression of *C. picta* deviates very little from the descriptions of the species found in manuals. The male flower consists of three stamens apparently attached directly to the

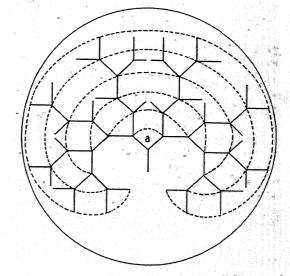


Fig. 4. A graphic representation of the dichotomously branching rhizome system. The solid lines represent the progressive development of the rhizome;  $\alpha$  is the angle formed by the first two primary branches, and the dotted lines represent expansion of the clump margin.

ixis of the inflorescence in a horizontal line. No rudiments of a perianth or gynoecium have been observed. Before anthesis the flower is covered by a subtending bract, and the 30 to 60 florets are aggregated spirally in a compact spike. The spikes occur singly on individual scapes (fig. 2).

The pistillate flower is as simple as the staminate but more difficult to interpret. It consists of a stipitate, tricarpellate pistil made up of a trigonous ovary, a slender style, and three filiform stigmas. There are no vestiges of stamens, or of a perianth.

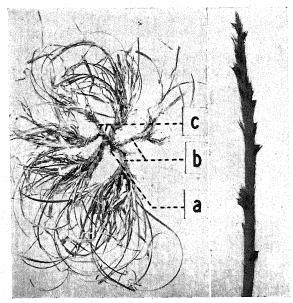


Fig. 5-6.—Fig. 5 (left). Rhizome system with roots removed; a, primary stem; b and c, secondary branching systems formed from first two branches.—Fig. 6 (right). Pistillate spikelets in a predominantly staminate inflorescence of Carex crinita.

Instead of being attached directly to the primary axis, however, the pistillate flowers are produced singly on short, inconspicuous, but definite secondary axes, which are located in the axils of spirally arranged bracts. Both the gynoecium and the dwarf axis are enclosed by bracteole, the margins of which are united forming a rather loose-fitting, sac-like structure with a small distal bidentate aperture. Since the pistil is much larger than its axis, this foliar envelope assumes the shape of the ovary, and the style and stigmas are exerted through the opening in its top. Inasmuch as the components within this floral unit are concealed by the subtending bract until maturity, the external appearance of the inflorescence (fig. 3) is similar to that of the staminate spike.

This axillary floret, consisting of a branch of limited growth, a bracteole, and a pistil, has been designated as a spikelet by several observers who have made morphological and anatomical studies of the Cariceae. Among the morphological investigations of *Carex* and related genera, the studies of

the bracteole have been especially prominent. According to Baillon (1894), it has been known under sixteen different names. At present it is known as the perigynium in American floras and as the utriculus (utricle) in most European literature. In this discussion the terms bracteole and perigynium may be used interchangeably. The former term, bracteole (Arber, 1925), however, applies to the first leaf on any branch of an inflorescence regardless of the order of the branch, while the term perigynium refers specifically to this special bracteole which accompanies each pistillate flower.

In many other species of Carex, where branching occurs in the reproductive shoot so that several spikes or spikelike inflorescences occur on a common axis, a bracteole is found sheathing the base of the peduncle of each inflorescence. Since more than one inflorescence is rarely found on a scape of C. picta, not many bracteoles of the latter type

have been available for observation.

FLORAL EXPRESSION.—Since stamens have long been associated with maleness and carpels with femaleness, diclinous flowers and dioecious plants have come to be known as male and female. This distribution of stamens and carpels in separate flowers is often followed with greater interest than when the flowers are perfect; and when bisexual flowers are found on typically monoecious or dioecious plants, or when sporophylls are observed with characteristics of both stamens and carpels, such plants and sporophylls are looked upon with special interest. At first these unusual conditions were described as teratological forms, but more recently they have been regarded as representatives of gradations in sexual expression. Many monoecious species have been pointed out (especially by Schaffner, 1921) in which sexual gradations of this type Similar observations (Schaffner, 1919; Yampolsky, 1920) have also illustrated bisexual tendencies in dioecious individuals. If these "abnormalities" represent intermediate sexual expressions, they may be conceived as common,2 although more frequent in some species than in others, and perhaps where they are thought not to occur, systematic studies will bring out an almost universal distribution among flowering species.

Within the genus Carex are a great many variations in sexual expression, and this unstable arrangement of staminate and pistillate flowers is known for both bicarpellate and tricarpellate forms. Investigators have pointed out this condition from time to time in discussing both monoecious and dioecious individuals. Holm (1921) found variations common enough in his Aeorastachyae, a group of monoecious Carices, to show the distribution of staminate and pistillate spikes by means of tables. One of the species constituting this group, C. crinita Lam., also illustrates especially well the irregular distribution of the two sexes in an individual in-

<sup>2</sup> Reference is made here to plants with diclinous flowers in which the two extremes in sexuality are characteristic, and not to the monoclinous where a bisexual expression is typical.

florescence. Figure 6, in agreement with Holm's description of floral expression in this Carex, brings out vividly one of the possible irregularities. In addition, Holm (1900) pointed out that sexual variation could be used as an aid in distinguishing C. circinata C. A. Mey from C. lejocarpa (leiocarpa) C. A. Mey when discussing the affinities of the two species. All specimens of C. circinata were found to be androgynous, while those of C. lejocarpa were found to be both dioecious and androgynous. It is also interesting that Kükenthal (1909) found it necessary to list monoecious varieties for many of the dioecious species which he recognized. Then Arwidsson (1928), after examining representatives of the Carex dioica-group, has discussed the prevalence of androgynous forms in Scandinavia as opposed to their rare occurrence in England. In contrast with this instability, it was noticed, when collecting inflorescences for morphological investigation, that C. picta was seemingly strictly dioecious. In order to secure data which would express the actual trend of sexuality in this species, the inflorescences of specific plants were examined during successive flowering periods.

This analysis of floral expression included 82 clumps, 79 unisexual and three mixed, the three mixed and 69 unisexual clumps being studied in the native habitat, and the remaining ten plants being grown inside and followed under greenhouse conditions

A very slightly grazed open woodland about two miles north of Bloomington, Indiana, was selected for field observations. In the spring of 1934, 23 clumps were plotted on a northwest-facing slope and 49 on a west-facing one. The ten clumps, five staminate and five pistillate, studied under artificial growing conditions were not transplanted to the University greenhouse until the following fall, and observations were not continued over as long a period as they were outdoors. Nevertheless, there were more data secured from each of these plants than were obtained from each plant in the native habitat, since those indoors flowered three to four times a year.

Each of the clumps of Carex selected in the native habitat in 1934 was marked with a metal marker, and floral analyses were continued during the flowering periods of the next four years. Thirty-two were originally recorded as purely staminate, 36 as pistillate, and three as mixed. The plants included in this survey, irrespective of sex, produced an average of 15-20 inflorescences each year. The number of inflorescences for both male and female plants, however, varied greatly. As many as 65 were recorded for one staminate clump, while 59 was the largest number found on a pistillate plant. The average number of inflorescences per staminate clump was consistently about one and a third times as large as that produced by the pistillate. It was also observed that plants of each sex did not produce inflorescences year after year without exception. Plants which failed to flower are referred to as "sterile."

Twenty-four (three-fourths) of the truly male clumps developed purely staminate inflorescences during all five seasons. Four staminate clumps were sterile one out of the five years. Three of this number were known to resume their original sexual expression; the fourth failed to flower during the final year of the survey. There were two male clumps for which data were incomplete: One of these was overlooked in 1936; in the case of the other the marker was "borrowed" in 1936 and not returned. Two other staminate clumps were found to produce an androgynous inflorescence. This type of inflorescence was observed on one of these plants in 1937 and in 1938. This plant was not an exceedingly vigorous one. In 1937 the androgynous inflorescence was the only one produced, but in 1938 four staminate inflorescences were produced in addition to the androgynous one. Each of the bisexual inflorescences of this clump was characterized by a single pistillate floret in the axil of the lowest bract. The other plant with the hermaphroditic tendency was observed with an androgynous inflorescence only in 1938. This plant, in contrast with the former, was vigorous and produced 42 staminate inflorescences in addition to the androgynous one. In this instance two pistillate spikelets occurred in the bisexual inflorescence, one in the axil of each of the two lowest bracts. One of the clumps, which was considered staminate during the first two years of the floral analysis, flowered as a mixed clump during the last three. At first it was thought that a partial reversal of sex had taken place. After careful observations, however, it was concluded that the clump was originally a mixed one and that the pistillate constituent had remained sterile during the first two years of the floral analysis.

Twenty-two of the clumps originally marked pistillate produced solely pistillate inflorescences from 1934 to 1938, inclusively. Thirteen of the pistillate clumps became sterile at some time during the five year survey. Ten of this number remained sterile for one year and then renewed production of the flowers originally recorded for them. The eleventh was sterile in 1936 and died before the next flowering season. The other two, having become sterile, failed to flower during two consecutive years. One of these produced pistillate inflorescences after the period of sterility; in the case of the other, the second sterile season came the last year of the floral analysis. One pistillate clump was overlooked in 1936, but during the remaining four years it was typically pistillate. Unlike the male plants analyzed, this group showed no sexual intergrades, neither gynaecandrous or androgynous (Mackenzie, 1931) inflorescences being observed in them during the survey.

The Carex plants making up the mixed clumps behaved in much the same way as did those growing independently. One of the three clumps produced

egularly male and female inflorescences each year; n another the pistillate constituent remained sterile n 1937 and then flowered in 1938. Since the third lump was not located in 1935, no datum is available for that year. In 1936 both of its components were sterile. During the last two years of the survey, female inflorescences were developed, but an expression of the male element was still not apparent.

The ten plants transplanted to the greenhouse grew well under these conditions. A change in environment and transplanting has been correlated with alteration of sexual expression in some plants,

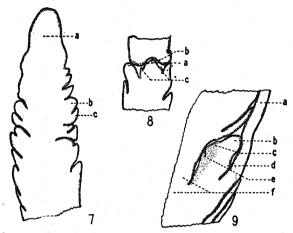


Fig. 7-9. Primordia of pistillate inflorescence.—Fig. 7. Tip of embryonic inflorescence in median longitudinal section showing: a, growing point; b, spikelet axis primordium; c, bract primordium.—Fig. 8. Tangential longitudinal section of embryonic spikelet; a, enclosing bract; b, axis of spikelet; c, collateral apices of perigynium.—Fig. 9. Median longitudinal section through embryonic spikelet; a, enclosing bract; b, axis of spikelet; c, gynoecium; d, abaxial level of perigynium; e, adaxial level of perigynium; e, main axis of inflorescence.

but, although in this instance the plants flowered three to four times a year, nothing of that nature was recorded. All the plants remained unisexual, and no bisexual inflorescences were observed.

From these observations, C. picta in this locality may be described as being remarkably uniform in its sexual expression, and the occurrence of the androgynous inflorescences brings out the fact that, although the species is remarkably constant in its sexual expression, it is not entirely without sexual intergradations.

The occurrence of sterility in the sequence of years is looked upon with interest. It, however, seemingly does not affect nor does it seem to indicate a reversal of sexual expression. That a change has taken place within the plant which upsets its potential sexual expression is realized. This condition, described by Schaffner as neutral, is undoubtedly not without significance, but in this species it could not be associated with the production

of androgynous inflorescences and sexual gradation.

ONTOGENY AND VARIATION IN FLORETS.-The interpretation of the relationship of the perigynium to the pistillate flower in Carex, as is presented in the discussion on the floral units of Carex picta, was first proposed by Kunth in 1835 (Eichler, 1875) after studying a species of Schoenoxiphium, and his explanation has received general support in the past century by such investigators as Townsend (1873), Eichler (1875), Pax (1885), Baillon (1894), Holm (1896), Kükenthal (1909), Snell (1936), and others. For the most part, they have stressed the evidence as brought out by the comparative morphology of the spikelets of Schoenoxiphium, Kobresia, Uncinia, and Carex, all genera of the tribe Cariceae. Townsend (1873), however, states that this same interpretation of the floral unit may be illustrated by deviations in the bracteoles of Carex glauca and possibly C. riparia. Later, Holm (1896) duplicates Townsend's explanation in citing transitions between the "ochrea" and the "utriculus" in Carex cladostachya Wahlbg. In addition to other illustrations within the genus, the cladoprophyllum and utriculus, as used by Kükenthal (1909) in the floral diagnosis of several species of Carex, are a notable supplement to the interpretations of Townsend and Holm. Then Snell (1936) has recently cited anatomical evidences along with a review of the explanations based upon comparative morphology.

The ontogeny of the spikelets of Carex picta brings out another interesting correlation with the explanations of the pistillate floret of Carex based on other evidence. A study of serial sections of this species shows, as early as the middle of August, embryonic inflorescences with the primordia of bracts and axillary axes near their tips. The arrangement and shape of these newly formed organs are typical for the beginnings of leaves and their axillary axes (fig. 7). Both are rounded on top, that of the bract being ridge-like and that of the axis being short columnar. Each primordium at first appears as a slight undulation. When that of the axillary structure is slightly longer than broad, the primordium of the perigynium becomes dis-The beginning of the perigynium is a cernible. collar-like ridge with two collateral prominences (fig. 8) which tend to slope downward adaxially and abaxially, the higher tangential edge usually being next to the bract primordium. Of the three initial structures the bract grows most rapidly. When the bract has grown to twice the height of the lateral axis, the primordium of the gynoecium is readily perceptible above that of the perigvnium on the side of the rachilla next to the rachis (fig. 9). The newly formed, obtusely hemispherical primordium is easily recognized by cells with extremely dense cytoplasmic contents. It soon becomes trilobate, each lobe representing a carpel. The lobes are arranged in a triangle with two transversely adjacent ones uppermost. Toward the end of Sep-

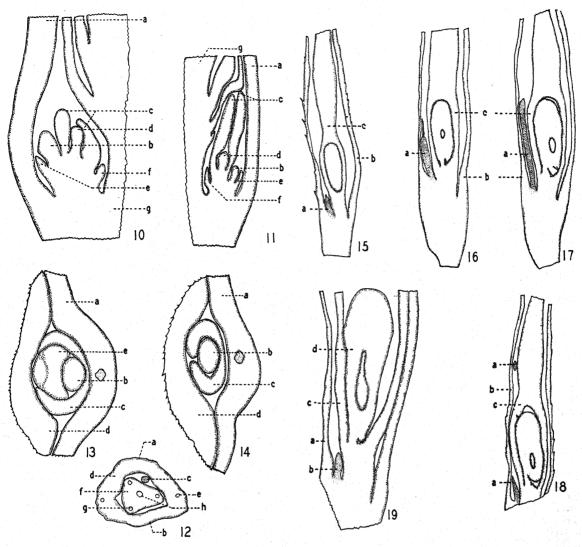


Fig. 10-19.—Fig. 10-11. Developing spikelets.—Fig. 10. Elongating primordia in median longitudinal section; a, enclosing bract; b, axis of spikelet (rachilla); c, carpels; d, ovule; e, abaxial edge of perigynium; f, adaxial edge of perigynium; and g, main axis of inflorescence.—Fig. 11. Young spikelet with nearly typical components in median longitudinal section, legend as for figure 10.—Fig. 12-14. Transections of spikelets.—Fig. 12. Orientation of components and conducting tissue at the base of a nearly mature pistillate spikelet; a, abaxial edge; b, adaxial edge; c, rachilla; d, perigynium; e, vascular strand of perigynium; f, base of ovary; g, vascular strand of carpel; h, vascular strand of ovule.—Fig. 13. Primordia of a fertile spikelet; a, bract; b, rachilla; c, perigynium; d, rachis; e, pistil.—Fig. 14. Sterile spikelet, legend as for figure 13.—Fig. 15-19. Variations in length of rachilla.—Fig. 15-18. Spikelets at maturity of embryo sac; a, rachilla (only base and apex showing in fig. 18); b, perigynium; c, pistil.—Fig. 19. Spikelet with maturing fruit; a, perigynium; b, rachilla; c, pericarp; d, immature seed.

tember the three embryonic carpels assume the upward course of growth taken by their parental axis. At this time the carpels and their axis (fig. 10) are about equally prominent, and the primordium of an ovule appears within the triangle formed by the carpels. The gynoecium develops rapidly, and usually by the middle of October it becomes several times as large as the rachilla (fig. 11), the upper portion of the columnar constituents elongating to form the style and stigmas, and the basal portions developing into the trigonous overy. Within the one-celled ovary, the basilary ovule now usually exhibits two integuments and a megaspore mother cell. At this age also the body of the perigynium envelops the major portion of the ovary, the collateral lobes rising slightly above it. The entire spikelet, however, remains covered by its subtending bract.

A transverse section through the base of a nearly mature pistillate floret (fig. 12) shows that the gynoecium has followed the course of development indicated in the embryonic stages. The vascular

strands and the lobes corresponding to the carpels show that one carpel is located next to the main axis of the inflorescence and two on the other side. The perigynium has two large vascular strands, which traverse the lateral lobes and aid in determining the orientation of the spikelet and its components.

The preceding has established the sequence in which the primordia of the parts of the pistillate floret appear in Carex picta. These primordia have been followed as they continued their development. Although the oldest examined are still immature, these spikelets appear to be characteristic for the species; and the succession and behavior of the primordia from which they are formed are in harmony with the interpretation of the pistillate floral units of the Cariceae, as has been established and maintained by other methods of investigation.

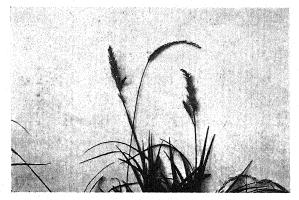


Fig. 20. Female inflorescences, one unbranched and two with lateral shoots at their bases.

Variations in the axes of the florets of *Carex* are frequently cited in the general literature on the genus, and the inflorescences of this dioecious species show variations which may be used to establish interesting correlations.

The pistillate spikelets thus far discussed have all been alike in possessing the morphological essentials of fertility (fig. 13). Whether or not fruits are ultimately formed is irrelevant. Besides these, there are spikelets with imperfectly formed flowers at the tips of inflorescences and sterile ones scattered among the fertile. Each of the latter, like the normal pistillate spikelet, has a short lateral axis of limited growth and is enclosed within a bracteole; the difference is that a gynoecium does not develop. A cross-section taken near the base of such a spikelet shows its axis encircled by the perigynium, but no trace of a pistillate flower is discernible (fig. 14). The fertile spikelets are numerous and prominent; the sterile are few in number, inconspicuous, and confined largely to the base of the inflorescence. A natural supposition is that the sterile spikelet is a modification of the pistillate in which the primordium of the pistillate flower has aborted.

Some other modifications, although less common, are equally interesting. It has been emphasized

that the axis of the pistillate spikelet aborts beyond the attachment of the flower. In C. picta the aborted tip is in most instances no longer than the stipe of the ovary (fig. 15). That this aborted terminus varies in length and function within a species of Carex has been known for some time. In 1873 Townsend made several general statements on its variable nature, calling it a seta. Two years later Eichler substantiated previous observations on the variability in the axis of the spikelet of C. microglochin, which had been described as often projecting above the perigynium and, under special treatment, even producing staminate flowers. More recently, abnormal pistillate spikelets with fertile stamens have been described for species of Carex, and rudiments of male florets have been observed along the tips of the axes of pistillate florets of some species.

In the study of serial sections of numerous spikelets of Carex picta, several interesting variations were found in the axes of the florets. Nothing comparable to a bisexual spikelet was found, but considerable variation in the length of the axes is apparent (fig. 15, 16, 17, 18). The spikelets were removed from the rachis and sectioned individually, and it was learned that when one spikelet of a specific inflorescence was found with an unusually long rachilla, the same would be true of many of the remaining ones. Whether or not this peculiarity was confined to a single inflorescence, or was characteristic of all the inflorescences of a given plant, was not determined. The longest axes observed (fig. 18) were almost twice the height of the developing

fruit.

Many of these axes, regardless of their length, exhibited a characteristically contracted terminal portion, which suggested that the aborted end had started to develop and then died and shriveled. An especially long terminus of this nature is illustrated in a spikelet with an axis of intermediate length (fig. 16). In most instances this rudiment apparently ceases to develop at about the time of the maturity of the embryo sac (fig. 15, 19).

Even more pronounced variations in the pistillate shoot have also been observed. Secondary branches, bearing spikelets like those on the main, usually unbranched axis, are sometimes found in the axils of bracts near the bases of inflorescences (fig. 20), but they are not at all as frequent as those variations detected with the microscope. Only five were observed, and four grew on plants in the greenhouse, three of the latter developing as a secondary growth in inflorescences far past their normal flowering period, and the other as the stigmas of the flowers of the main axis began to die. All of the components of the one branched inflorescence found in the natural habitat were formed at approximately the same time.

These lateral shoots were found to have characteristics in common with the pistillate spikelets. The base of each was surrounded by a bracteole (fig. 21), and each arose from the axil of a bract

similar to that which subtended the spikelets. If the lateral shoots were permitted to remain attached until fruits matured, the entire shoot fell from the inflorescence, a characteristic common to individual spikelets. The conclusion naturally follows that these long branches have developed from the axes of spikelets which did not abort in the usual manner.

The variable character of the bracteoles enveloping the basal portions of the long branches deserves additional comment. In the case of the secondgrowth branches which developed in the greenhouse, the bracteoles were of the normal bidentate form, while on the one branch which developed simultaneously with the fertile spikelets, out of doors, the bracteole was split down the abaxial side. That is, the bracteole, having played the rôle of a perigynium previous to the development of the branch, still portrays the bidentate character, while that which developed simultaneously with its axis was split down the abaxial side. The open bracteole is more like the typical monocotylous prophyll, while the closed ones substantiate clearly the assumption that the axis of a spikelet is in reality an aborted floral branch. The variations in these bracteoles provide good additional evidence as to the probable ontogeny and phylogeny of the perigynium.

The staminate floral unit, because of its simplicity, has always been considered self-explanatory, and the many discussions on Carex florets have centered around the pistillate unit. Classification of the perigynium as a specific type of floral component and then determining it as a simple or compound structure have been interesting and significant subjects for discussion and investigation; in the end they have disclosed the morphological nature of the floret. On the other hand, the staminate unit has received less attention, probably because it is thought to be easily understood. This apparent simplicity may, however, obscure an intangible complexity.

A clue to the morphological nature of this floral unit is found in an abnormal staminate floret of Carex acuta L. mentioned by Boott (Holm, 1896). This floret, as illustrated, has the stamens enclosed in a perigynium. The perigynium, as associated with the pistillate floret, necessitates the presence of a rachilla. As has been stated, neither a secondary axis nor a bracteole is associated with a typical staminate floret, and their existence in this instance suggests that, phylogenetically speaking, they might have been present in a previous form. We may assume, then, that the male spikelet as it exists is now highly specialized, the bracteole and its axis having been reduced to mere potentialities, and the three characteristic stamens arising from the hypothetical axis.

To test this assumption, a study has been made of the ontogeny and variations in the male floret. Serial sections have been made of spikelets of all ages from the time of the appearance of the first primordia to the full development of the typical

components. In the embryonic male flower bud collected during August, the beginnings of the staminate spikelet, a bract primordium and an axillary axis, are in evidence at the tip of the inflorescence. The former is short, rounded, and ridgelike, the latter hemispherical. Both appear like the two initial primordia of the female inflorescence. Each develops rapidly, the bract primordium soon covering the axillary. From the latter, three

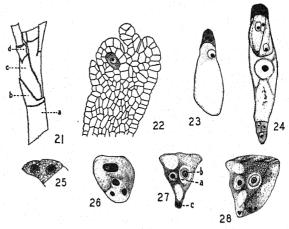


Fig. 21–28.—Fig. 21. Junction of lateral shoot and ma'n axis a, scape; b, subtending bract partly cut away to show bracteole; c, bracteole; d, axis of secondary shoot.—Fig. 22. Bending ovule showing megaspore mother cell, beginning of inner integument on both sides of nucellus, and initial of outer integument on right of nucellus.—Fig. 23–24. Development of embryo sac.—Fig. 23. One megaspore enlarged and three disintegrated.—Fig. 24. Seven-celled embryo sac and remnants of three disintegrated megaspores.—Fig. 25–28. Normal development of pollen grain.—Fig. 25. Two microspore mother cells.—Fig. 26. Tetrad of "microspores."—Fig. 27. Pollen grain in two dimensions; a, tube nucleus; b, generative cell; c, three disintegrated "microspores."—Fig. 28. Cuneate pollen grain in three dimensions.

slightly adaxial terminal lobes appear when the primordium has become scarcely longer than broad. These lobes mature rapidly into three stamens, and their basal beginning becomes scarcely perceptible.

The initial stages in the development of the staminate spikelet add little support to the assumption that this floral unit once possessed the constituents of the pistillate floret. The similarity of the first two primordia of both spikelets might be considered favorable evidence, but little emphasis should be placed upon it, since the sequence of these primordia is typical for leaves and axillary axes in general. In this the observation, however, does support the idea of the development of the stamens of the male flower from a rudimentary rachilla rather than directly from the rachis of the inflorescence. Their apparently adaxial development is of problematic importance.

Additional support favoring the development of the staminate flowers from a secondary aborted axis is found in the occurrence of typical pistillate florets at the base of a staminate inflorescence. Such a condition is abnormal for the species, but it is significant because a female floret has developed in a position normally occupied by a male. It has been definitely ascertained that the female flower develops from a secondary axis, and it is probable that the same holds true for the male. The development of a more elaborate floral unit in the position of one with fewer components makes it appear as if the two may have a common origin. This is in agreement with ontogenetic observations of the two units. It follows, then, that when a pistillate floret replaces a staminate one, the primordium of the floret undergoes a transition which Schaffner has called a change of "functional activity." In the one instance, a condition prevails which limits the development of the secondary floral axis to the production of a single flower, three stamens, before it aborts; in the other, a condition occurs which promotes the development of a flower and its subtending bract, the perigynium, on an axis which is plainly evident beyond the place where the flower

In the literature it is suggested that the pistillate flowers of Carex develop upon secondary axes, the staminate on the primary axis. The evidence furnished by a comparative study of the development and variations of the pistillate and staminate florets of C. picta suggests that the two kinds of florets represent axes of the same rank which differ in their degrees of development. Either type of axis is easily overlooked in floral analysis. That of the pistillate floret is the more easily recognized, but may require a microscope. The rachilla of the male floret is poorly developed and is detected only during early stages of development. It is not unusual for the axis of the female unit to vary in length, and these variations clarify its identity. Variations were not observed in the axes of male florets, and, when an axis on which a male flower is expected to develop becomes longer than is typical for the staminate unit, it apparently changes its functional activity, and a pistillate flower is produced.

Gametogenesis.—In connection with the study of floral expression and floral development, the following observations were made on gametogenesis. The development of both gametophytes is much the same as Heilborn (1918) has described for some other species of Carex. Since no dioecious species were included in his discussion, however, and since there appear to be some slight differences between Carex picta and the vernal species with which he dealt, descriptions of male and female gametophytes are of interest.

The observations on megagametogenesis were made from slides prepared for studying the ontogeny of the female spikelet. Several fixing reagents and staining combinations were used with success, and the sections were cut at various thicknesses ranging from 5 to 15 microns.

Normally a solitary cauline ovule arises in the bottom of the one-celled ovary, and, before differentiation takes place, the ovule primordium stands as a straight, short-cylindrical organ (fig. 10, d). As a centrally located hypodermal cell, the archesporial cell, enlarges and divides anticlinally forming a parietal cell and a megaspore mother cell, the ovule begins to bend toward the rachis, and the inner integument appears.

This differentiation, in Carex picta, takes place in the fall, while in those vernal species investigated by Heilborn (1918), the archesporial cell did not develop until the following spring. female spore mother cell in C. picta, which is often differentiated in September, enlarges slowly, but does not undergo division until a few days before The parietal cell soon divides perianthesis. clinally, and the initial of the outer integument becomes apparent in a short time (fig. 22); but other changes in the ovule follow slowly. As the bending ovule gradually turns over, the integuments form a double jacket of tissue over the nucellus, and the cells adjoining the megaspore mother cell undergo a few divisions. The tissue between the megaspore mother cell and the epidermis, however, seldom becomes more than two cells in thickness before maturation. This is another exception to what Heilborn found. He described the spore mother cells as becoming more deeply embedded, and the same is shown in illustrations by Wahl.

Frequently, as is characteristic of anatropus ovules, the outer integument fails to develop on the side of the ovule which is being folded back upon the funiculus, and this folding may also affect the uniform development of nucellar tissue, growth being suppressed adjacent to the region where the ovule pushes against the ovary wall. Usually during March of the following year, the ovule rapidly increases in size, and the "normal-type" of embryo sac (Schnarf, 1936) develops from the megaspore mother cell within a few days. The details of the formation of the linear tetrad from the mother cell were not observed, but that the chalazal megaspore is the one to germinate is definitely indicated in sections of the ovule made at any time between the first steps in the germination of the megaspore and the maturity of the embryo sac (fig. 23, 24).

It has been known for some time that, as a rule, in *Carex* only one pollen grain develops from a microspore mother cell, but it was considered worth while to investigate this point in a dioecious species. Both serial sections and permanent smears were used in this part of the study.

Cross-sections of anthers show no unusual features. The slender, deeply four-lobed anthers dehisce along two collateral sutures, the pollen developing in four long, slender, cylindrical sporangia. The sporogenous tissue is differentiated in

<sup>3</sup> Illustrations on megasporogenesis (Hill, Overholts, and Popp).

September and October, but the microspore mother cells (fig. 25) do not undergo division until immediately preceding anthesis; so rapid is the formation of pollen from the mother cells that the stamens of one floret of an inflorescence may contain mature pollen while the fertile tissue of an adjoining one is still in the spore mother cell stage.

The nucleus of the mother cell usually gives rise to four nuclei4 (fig. 26), but in some instances one or two additional divisions occur so that more than four nuclei are formed. However, in most cases observed, all except one of these remained at the bottom of the cuneate mother cell. After short time the latter disintegrate and become very inconspicuous. The persisting nucleus migrates toward the center of the cell and typically divides immediately to produce a tube cell and generative cell (fig. 27, 28). In the usual form of microsporogenesis four spores are developed from a mother cell, and an equal number of pollen grains are produced. Species of Carex then produce only one-fourth as much pollen, in proportion to the number of microspore mother cells present, as is formed by most plants.

Pollen grains germinated on a solution of 2.5 per cent cane sugar and 1 per cent agar produced tubes from lateral germ pores and from the large end of the grain (fig. 29, 30, 31). In a few instances two tubes grew from a pollen grain (fig. 31). This was of more than usual interest, as the germination of the persisting microspore was at times followed with difficulty (fig. 32, 33) and in a few cases it appeared as if two tube nuclei and two generative cells had developed in a pollen grain. It is likely that grains of this nature are the ones to develop more than one pollen tube.

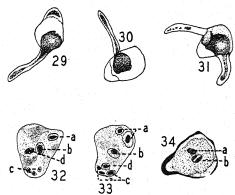


Fig. 29-34. Germinating pollen grains.—Fig. 29. Pollen tube coming from enlarged end of pollen grain.—Fig. 30. Pollen tube coming from side of pollen grain.—Fig. 31. Two pollen tubes from one pollen grain.—Fig. 32-33. Pollen grains with more than typical number of cells; a, tube nucleus; b, generative cell; c, disintegrated "microspores"; d, probable second generative cell having divided in figure 32.—Fig. 34. Normal pollen grain; a, tube nucleus; b, generative cell having divided; and remnants of disintegrating "spores" not perceptible.

\* In some instances these nuclei appeared to develop a wall, while in others they did not.

The generative cell was observed to divide before the pollen tube developed (fig. 34), but the nature of this division, and the behavior of the male gametes and tube nucleus in the pollen tube have not been worked out. Fertilization was not observed, but pollen tubes were found to reach the ovule 8 to 12 hours after pollination. Plants are often ready for pollination by the middle of March in our latitude, a time subject to radical changes in temperature, and there may be considerable differences in the length of the interval required for the pollen tube to complete its growth.

Incidentally, chromosomes were observed in microsporogenesis of one flower. Counts were made from longitudinal serial sections cut 10 microns thick and stained with the crystal violet chromosome stain. Most of the pollen mother cells in each of the three anthers were undergoing maturation. The chromosomes studied were in the metaphase of the first division; and, from ten cells suitably oriented, the zygotic number seems to be 32.

### SUMMARY

Floral analyses show that the sexual expression in Carex picta is remarkably uniform, and the occurrence of an occasional androgynous inflorescence corresponds to a condition known for other species described as dioecious.

The sequence of the primordia in the development of the pistillate inflorescence shows that the pistillate "flower" of this species is actually a floral unit, and the true female flower in the form of an unattended pistil is attached to a rudimentary rachilla, both pistil and rachilla being surrounded by a bracteole. This interpretation is not new for species of Carex; but it has heretofore been based upon other evidence.

Variations in the length of the female floret axis occur in Carex picta, as have been found for other Carices. A part of these variations of the rachilla exist within the perigynium, but a lateral shoot resembling the usual solitary inflorescence may develop from the rachilla of a basal spikelet. These variations also aid in explaining the morphological nature of the female floret.

The apparently self-explanatory staminate floral unit also develops upon a secondary rudimentary axis and seems homologous to the pistillate, its apparent simplicity being due to its highly evolved nature.

In megasporogenesis a hypodermal archesporial cell is differentiated and divides, giving rise to a megaspore mother cell early in the autumn preceding anthesis. According to the literature, in other vernal species this differentiation does not take place until the following spring.

Nucellar tissue before maturation is not as abundant as has been found in the ovules of other Carices; however, the anatropous ovule and the development of the common type seven-celled embryo

sac from the chalazal megaspore agrees with descriptions for other species.

Microsporogenesis is typical for most sedges, but it is unusual for flowering plants in general in that only one of the "spores" resulting from maturation develops into a pollen grain. It is also unusual to have the wall of the spore mother cell become the wall of the pollen grain.

The generative cell was found to divide before a pollen tube developed. Pollen tubes usually reach the embryo sac within 12 hours after pollinaPollen tubes grow from the broad end or from the sides of the cuneate pollen grain.

In some instances two pollen tubes grow from a single pollen grain, and the occasional presence of more than two apparently functional cells in developing pollen grains is logically associated with the development of two pollen tubes from one grain.

The zygotic number of chromosomes seems to be 32.

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## SOME PHYSIOLOGICAL CHARACTERISTICS OF MYXOMYCETE SWARM-CELLS 1

## Paul E. Kambly

Since Harper (1900) discussed nuclear fusion in Fuligo septica and Pinoy (1908) postulated + and — amoebae of Didymium nigripes there have been several studies of "sexual" phenomena in the life histories of Myxomycetes. Most of these studies have been concerned with the interpretation of nuclear changes. Monoploid and diploid phases have been discussed by Jahn (1911, 1936), Skupienski (1927), Wilson and Cadman (1928), Cayley (1929), Schünemann (1930), Howard (1931) and von Stosch (1935, 1937). In contrast with these papers on nuclear phenomena in the life histories of Myxomycetes, Abe (1934) made a study of certain physiological characteristics of Myxomycete swarm-cells, using Fuligo septica, Erionema

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aureum, Didymium nigripes, Physarum crateriforme and Stemonitis fusca. She found that there was a fusion of swarm-cells in all these species. She observed accompanying karyogamy in Fuligo septica and Erionema aureum. Since in every case the fusing cells were isogamous, she called the cell which "flowed" into the other the "male." Beginning with this assumption of "male" and "female" swarm cells, she performed a series of physiological experiments designed to discover differences in these two lines. On the basis of the reactions of swarm-cells to indicators and other chemical compounds, she concluded that there is no difference in their pH; that the "male" has a higher oxidationreduction potential than the "female"; and that the "female" has a positive electrical charge and the "male" a negative charge.

A review of the literature reveals that no other work of similar nature has been reported for the swarm-cells of Myxomycetes. Since the clear-cut differences which Abe describes between the members of a fusing pair of swarm-cells would be of considerable significance in explaining the phenomena of sex in lower organisms an attempt was made to substantiate her findings. This paper is a report of a series of experiments designed to study the pH, the reducing intensity, the surface charges of swarm-cells, and the effects of vital stains and toxic ions on them.

MATERIALS AND METHODS.—Reticularia Lycoperdon was chosen for study because 85-95 per cent of the spores germinate in distilled water in less than an hour and because the details of its cytology have been reported by Wilson and Cadman. The protoplast which emerges from a Reticularia spore soon develops its swarm-cell characteristics and retains these characteristics until fusion. Very often these swarm-cell characteristics are observed in the zygote. Except in a few late checks, all Reticularia spores used were from a collection made at Iowa City on September 10, 1934. The swarmcells of Enteridium Rozeanum (coll. Nov. 18. 1934), Stemonitis Webberi (coll. July 28, 1934), and Fuligo septica (coll. June 27, 1936) were used at different stages in the study to check the results obtained with Reticularia.

All spores used in these experiments were germinated in ordinary distilled water in Syracuse watch glasses at room temperatures varying from 20°C. to 27°C. The pH of this water changed with ageing cultures so that in three days it was approximately 5.2. When the effects of dyes or indicators were studied, a drop of the culture was transferred to a depression slide and a small amount of the reagent added. Observations were made with a 10× objective and a 3 mm. Spencer water immersion lens. A Bausch and Lomb substage lamp furnished the source of light. Special methods and materials used in specific experiments will be described in connection with the discussion of these experiments.

EFFECTS OF CERTAIN VITAL STAINS.—Since Wilson and Cadman had reported that a number of mitotic divisions took place before swarm-cells were able to fuse, it seemed possible that the mitotic divisions were accompanied by physiological changes which resulted in the ability to fuse. For this reason the effect of vital stains on the swarm-cells taken from cultures of varying ages were studied.

Methylene blue, neutral violet, neutral red and Janus green were used in 0.01 per cent aqueous solution. The results obtained were not consistent. If the swarm-cells were left in the stain long enough, it was usually possible to make observations at a time when approximately 50 per cent were unstained. The heavily stained cells were granular in appearance and probably dead. If the concentration of the stain was lowered to the point where the swarm-cells would remain active, no difference in staining reaction was evident. In every case where fusing cells were observed, both members remained unstained at least as long as they

were active. In one instance a drop taken from a 36 hour culture was placed in 0.1 per cent methylene blue and three fusing pairs were observed. All three pairs remained colorless while many spherical cysts in the same field became intensely blue. Very often the stains caused the spherical swarm-cells or zygotes to swell and finally to burst. The more fluid portion of the cytoplasm flowed out through the ruptured cell-membrane leaving the stained granules behind. This produced what appeared to be two connected cells one of which was heavily stained and the other unstained.

Abe reported that swarm-cells killed in osmic acid vapor and then placed in the dyes showed a differential stain similar to that which she obtained with living cells. This result was confirmed but the question arises as to whether this difference in staining reaction is due to physiological activities which cease with death of the cell or whether these differences could be explained on other grounds. Further tests, in part similar to Abe's, were made, therefore, in an attempt to discover whether there were not other factors which would aid in explaining the presence of stained and unstained swarm-cells in the cases where it did occur as described in the above paragraph.

PH of swarm-cells.—Small's (1929) range indicator method of studying pH was used. With one exception indicators in both 10 per cent alcohol and in aqueous solution were made up in concentrations of 0.01 to 0.04 per cent. Di-ethyl red was made up only from an aqueous solution. The swarm-cells were left in the indicators until a uniform color was evident, the time varying from a few minutes to ten hours. Motility of flagellated swarm-cells is an obvious evidence of life. In most of the tests the flagella were retracted and cysts formed before any color was visible, so that it is probable that the color reactions obtained were in dead cells. This raises the question as to how much the pH of the cell contents changes on death.

In discussing this method of determining pH Small says, "The many times repeated tests had shown that there were no color differences between alcoholic and aqueous indicators, and that there were no color differences between periods of immersion of one hour to twenty-four hours. . . . It, therefore, appears reasonably certain that the observations made quickly are, in most cases, made upon living cells, while the later observations are made upon dead cells. The comparative tests indicate that the later observations can as a rule be taken as giving the initial pH of the living cells when the R. I. M. is used. With 'tint' indicator methods the results would be too complicated by this factor to be at all reliable." If Small's findings on the cells of higher plants are applicable to swarm-cells, then the results shown in table 1 are valid.

Except in the tests with methyl and di-ethyl red the colors obtained were very evident. The swarmcells appeared very faintly yellow in these two

Table 1. pH value of swarm-cells.

Indicator	Conc.	Alk. color range	pН	Acid color range	pН	Color of swarm- cells
Brom-thymol blue						
(Dibromothymolsulfonphthalein)	0.04	Blue	7.6	Yellow	6.0	Yellow
Brom-cresol purple						
(Dibromo-o-cresolsulfonphthalein)	0.04	Purple	6.8	Yellow	5.2	Yellow
Di-ethyl red						
(o-carboxybenzene-azo-diethyl-aniline)	0.04	Yellow	5.9	Red	5.6	Yellow
Methyl red						
(o-carboxybenzene-azo-dimethyl-aniline)	0.03	Yellow	5.6	Red	5.2	Yellow
Benzene-azo-a-naphthylamine	0.01	Yellow	4.8	Red	4.4	Yellow
Brom-cresol green						
(Tetrabromo-m. cresolsulfonphthalein)	0.04	Blue	4.4	Yellow	4.0	Blue

indicators. Benzene-azo-a naphthylamine was the only indicator which gave an immediate uniform color reaction in the swarm-cells. It should be pointed out that when using brom-thymol blue, brom-cresol purple and brom-cresol green, it was possible to make observations at a time when the swarm-cells were not uniformly colored and on the basis of those observations conclude that the pH of the cells differed. When they were left in the indicators as advocated by Small, a uniform coloration resulted which indicated a pH of approximately 6.

REDUCING INTENSITY OF SWARM-CELLS.—Table 2 shows the results obtained with a series of oxidation-reduction indicators. All the indicators were used in studying suspensions of *Reticularia* swarm-cells. They were added to 2 cc. of suspension in distilled water to make approximately a M/10,000 concentration in each vial. The effect of the indicators on the color of the swarm-cells was determined by observation with the water immersion lens. The tests were made at room temperatures varying from 22°C. to 26°C.

Table 2. Results with oxidation-reduction indicators.

Indicator	Color of swarm-cells in indicator	Effect of suspension on indicator
Phenol indophenol	Colorless	Reduced
0—cresol indophenol 1—naphthol—2—	Colorless	Reduced
sulfonate indophenol	Colorless	Reduced
Brilliant cresyl blue	Colorless	Reduced
Gallocyanin	Colorless	Reduced
Methylene blue Indigo tetrasulfonate Methyl capri blue nitrate	Some blue Some colorless Colorless Colorless	Not reduced Not reduced Not reduced
Indigo trisulfonate	Colorless	Not reduced
Indigo disulfonate	Colorless	Not reduced
Indigo monosulfonate	Colorless	Not reduced
Phenosafranine	Red	Not reduced
Neutral red	Some red Some colorless	Not reduced

On the basis of the results given in table 2, methylene blue and neutral red are the only indicators which give any evidence of two "lines" of swarmcells. In order to recheck this phenomenon of differential staining in methylene blue, a solution of LaMotte special methylene blue was used. In a M/10,000 solution of this indicator occasional blue quiescent swarm-cells appeared within ten minutes, and if left several hours practically all became blue. If the swarm-cells were first killed in osmic acid vapor and then placed in the indicator, they became uniformly blue within ten minutes. If this special methylene blue failed to stain the majority of swarm-cells because of their reducing intensity, then killing the cells in osmic acid vapor should result in their staining blue upon being placed in the indicator. Since this was the result obtained, the question arises as to whether those swarm-cells which were quickly stained blue in living cultures became colored because of a difference in reducing intensity or because they were dead. The latter seems to be the obvious answer.

Hewitt (1931) lists the errors involved in the use of oxidation-reduction indicators as follows: "(1) Damage to the cell by the dye or by injection. Although the cell may survive, the injury may have altered the electrode potential. Either the oxidised or reduced form of the dye, or both, may be toxic. (2) The dye may catalyse biological oxidations (or act as oxygen carrier) and hence alter equilibrium. (3) The capacity of the dye system may be so large, compared with the biological system, that equilibrium may be disturbed, and the time involved for the reattainment of equilibrium may be very long. (4) The properties of the dye may be altered by combination with cellular contents, etc."

The chief object in studying the reducing intensity was to determine whether swarm-cells differed in this respect as stated by Abe. Since this did not involve an accurate quantitative determination, some of the sources of error listed above need not be considered. Since the results obtained indicate a uniform reducing intensity, they are not in accord with Abe's conclusion.

Effects of toxic ions on swarm-cells.—In discussing this topic Abe wrote, "If gametes are negatively charged, they must absorb cathion, and if positively charged, anion. In order to see whether the gametes show any difference in resistance against toxic ions or not, the writer [Abe] tested the action of CuSO<sub>4</sub> and KCN at several concentrations. In 0.03 mol solution of CuSO<sub>4</sub>, half of the gametes of Fuligo septica and Didymium nigripes at once coagulated and sank to the bottom, while the other half continued the movement for a few minutes. The gametes were separated into two parts by sucking up the upper part of the gametes with a pipette. By adding 0.01 per cent solution of neutral red or methylene blue to each of these two portions, the coagulated gametes were stained red or blue, while the other gametes became vellowish. In 0.03 mol solution of KCN for ten minutes some of the gametes stopped the movement and become spherical, while the others continued the movement in the comma-shaped form. Gametes are fixed in such a state in the vapour of osmic acid for 30 seconds. Staining them with a very weak solution of methylene blue we find that the comma-shaped gamete appears blue, while the spherical gametes appear yellowish blue. We may come to the conclusion that the male gamete is less resistant against CuSO4 than the female, and vice versa against KCN. In other words, the cathion (Cu<sup>++</sup>) acted on the male gamete, and the anion (CN-) on the female gamete, so that it may be said that the male gamete is negatively charged and the female gamete is positively charged.'

M/300 copper sulphate killed some swarm-cells more quickly than it did others. Those which rounded up first settled to the bottom of the watch glass and could be removed with a pipette. These coagulated cells became blue when placed in 0.01 per cent methylene blue much more rapidly than those which were still active. This is in accord with Abe's findings.

Abe's results with potassium cyanide could not be repeated. Swarm-cells left in M/300 potassium cyanide and then killed in osmic acid vapor became uniformly blue in dilute methylene blue.

The fact that the swarm-cells which died first when placed in M/800 copper sulphate became blue when placed in 0.01 per cent methylene blue was not surprising, because they had the same coarse granular appearance of the stained cells discussed under vital staining. If a group of organisms placed in an unfavorable environment is observed at the proper time, it will be evident that approximately 50 per cent are affected by the change in environment and 50 per cent are unaffected.

If conclusions concerning the electrical charge borne by swarm-cells are to be drawn from experimental work with toxic compounds such as copper sulphate and potassium cyanide, it will be necessary to demonstrate a relationship between electrical charge and toxicity. KCN may be toxic by virtue of the undissociated HCN molecules which penetrate the cell, rather than by its CN-ion. It seems fallacious to conclude that organisms which are first affected by these toxic compounds are affected because they bear a different charge than similar organisms which are able to withstand the toxic effects for a slightly longer period.

Surface charge of swarm-cells.—Abe's conclusions concerning electrical charge were based in part upon experiments with potassium ferrocyanide and iron chloride. She placed swarm-cells in 10 per cent solution of potassium ferrocyanide on a glass slide and evaporated the fluid over a flame. After washing with water, a 10 per cent solution of iron chloride was added to the slide. She states that, "Some of the gametes were stained greenish blue, while others remained unstained, so that the former is considered to be female, having positive charge, and the latter male, having negative charge."

When swarm-cells were placed in 10 per cent potassium ferrocyanide on a glass slide and the solution evaporated over a flame, the cells shrunk excessively and were difficult to distinguish in the mass of crystals. When 10 per cent iron chloride was added, it was impossible to distinguish two lines of differently stained swarm-cells as described by Abe. Using the same solutions and centrifuging the swarm-cells through the potassium ferrocyanide and water also yielded poor results because of the shrinking of the swarm-cells. There were some spherical forms but none were present which were blue in color. It was found that when the concentration of the potassium ferrocyanide was lowered to 5 per cent the swarm-cells rounded up but did not shrink. These cells were washed through several changes of distilled water by centrifuging and then removed with a pipette and added to 10 per cent iron chloride. These were observed with the water immersion lens and were uniformly lightly stained. This was true of swarm-cells taken from cultures of varying ages.

These results did not substantiate Abe's findings. Lowering the concentration of the potassium ferrocyanide should not inhibit the adsorption or entrance of the Fe(CN)<sub>6</sub> ions into swarm-cells if the entrance is due to the electrical charge as she postulated. Since a uniform pale blue to colorless reaction was obtained with the lowered concentration, it is doubtful if her interpretation was correct. Her results suggest that the swarm-cells subjected to the extremely toxic concentration were affected in different degrees and because of greater or less entrance of ions showed a difference in intensity of staining. This is exactly what one would expect if observations were made at the proper stage of the experiment.

The electrical charge borne by the swarm-cells within the pH range of the distilled water used in

these experiments was determined by observing their electrophoretic movements. An electrophoretic cell was built by cementing two glass slides to a flat glass plate. The ends of this cell were sealed with agar. The electrodes used consisted of glass tubes with flared and flattened ends plugged with agar which was prepared with distilled water. A concentrated zinc-sulfate solution was placed in the tubes and zinc strips were immersed in the solution. The agar plugs in the flared ends of the electrodes made contact with the agar ends of the cell. The agar parts were freshly made each time the cell and electrodes were used. Dry cells were used as a source of potential.

When a suspension of swarm-cells was placed in this cell, it was sealed over with a coverglass and vaseline. In a field with a potential drop of approximately one volt per centimeter the swarmcells, cysts and zygotes moved uniformly toward the positive electrode. When the current was reversed, the direction of migration was immediately reversed. This was true of swarm-cells of various ages. In no case was there migration of all or part of the cells toward the negative electrode at any level in the cell.

Since migration in an electrical field is due to surface charges and these charges may have nothing to do with staining reaction, no definite conclusions concerning staining reaction can be drawn from the described mass migration toward the anode. The uniform migration does indicate that if swarmcells react to vital stains differently because of opposite electrical charges the presence of these charges cannot be detected by the method described.

#### SUMMARY

The swarm-cells of Reticularia Lycoperdon were studied in an effort to discover physiological factors which are associated with the ability to copulate. This paper is a report of experiments to determine the pH, the reducing intensity, the surface charges of swarm-cells, and the effects of vital stains and toxic ions on them.

When placed in certain vital stains, all swarmcells did not stain uniformly or at the same rate. Very often there were many heavily stained cysts and cysts which were not stained.

As determined by Small's range indicator method, the pH of the swarm-cells was found to be approximately 6.

Oxidation-reduction indicators revealed no definite difference in reducing intensity between swarmcells of the same age or of different ages.

Experiments with copper sulphate, potassium cyanide, potassium ferrocyanide, and iron chloride did not support the view that there are oppositely charged swarm-cells which copulate partly because of an opposite electrical charge.

Using an electrophoretic cell, the surface charge borne by swarm-cells, cysts, and zygotes was found to be negative. There was no evidence of positively charged swarm-cells at any age or at different levels of the cell.

The difference in staining reaction in vital stains was probably not due either to a difference in reducing intensity or electric charges as postulated by Abe.

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## THE MORPHOLOGY AND EMBRYOGENY OF SEQUOIA GIGANTEA <sup>1</sup>

J. T. Buchholz

WHEN ONE considers the difficulties encountered in collecting material for a study of Sequoia gigantea, he comes to appreciate one of the reasons why this species has been neglected by morphologists. In cultivation at lower altitudes the ovules and seeds of the cones that may occasionally be found are usually abortive. In the mountainous region of the Sierras where this species is native and reproductive, the trees are so large that one cannot hope to obtain cones from them without expensive equipment and preparation. The fact that the cones persist on the trees for many years after the seeds are mature (Buchholz, 1937, 1938a) has not been generally understood and adds to the difficulties confronting the collector. To be sure, squirrels often cut off cones which may be picked up under the trees, but these are usually the older ones. Various individuals have collected and sent me cones at different times during the past twenty years. cones received were found to be fully matured.

During the spring and summer of 1936 the writer went to California for the purpose of making a study of the Sequoias. The only practical plan worked out at that time which would not entail expensive equipment was to use the occasional cones produced on the small second-growth trees. Trees less than 75 years old are abundant in Whitaker Forest, a preserve owned by the University of California near Redwood Canyon. They are also abundant in the cut-over region near General Grant National Park.

During late June and early July while the new cones are still only partially grown, they may be recognized with field glasses by their smaller size and lighter color. It was found (Buchholz, 1938a) that while only one tree in several hundred in this second-growth stand may bear one or two cones (always near its top), they may be marked and mapped for collections during the summer when the cones are in the desired stage. About eight such trees were found, and the cones collected from them furnished me with the material for a study of the

embryogeny.

The stage of fertilization was not included. The cone collected nearest that date happened to be a teratologically misshapen specimen containing very few normal ovules. Some of the other collections were spaced a little too far apart in time, but the cones obtained were excellent and yielded an abundance of embryological material in their respective stages both before and after fertilization.

It was very fortunate and timely for my work that Looby and Doyle (1937) recently published their account of fertilization and the proembryo from material obtained in cultivation in Ireland. Had the writer planned a cooperative research with these investigators so as to pool all efforts in a study of the embryogeny of the big tree, a more

<sup>1</sup> Received for publication December 12, 1938.

precise division of labor could not have been outlined. Their account has supplied the stages missed by the author and enables him to describe the embryogeny with a satisfactory understanding of the proembryonic stages.

It is of great importance to know that the proembryo of Sequoia gigantea has a free nuclear stage and to know how this proembryo is organized. The younger stages observed by me and shown in figures 23-25 represent the stage in which Looby and Doyle left off in their account. Thus, it is now possible to give a fairly complete general outline of the early embryogeny of this species.

The ovules were collected and killed in Nawaschin's solution and in formalin-acetic-alcohol. The latter killing solution was found more satisfactory on the whole. Some of the material was cut serially in paraffin and stained by the usual methods. Nearly all the material that was ultimately found useful in a study of the later embryo was dissected material, the preparation of which has been described in considerable detail elsewhere (Buchholz, 1938b). For the last stages before fertilization and all subsequent stages, the seed coats must be removed; otherwise the seeds cannot be cut, because there is hardened tannin jell in the cells. The nucellus is thin and may be cut in paraffin without difficulty. Sections were cut in longitudinal axis and transversely. The most satisfactory orientations for cutting were those giving transverse serial sections of the nucellus.

The literature on the morphology of Sequoia gigantea is very meager. Strasburger (1872, 1879) described the cones of S. sempervirens, confining himself very largely to external morphological features and the vascular supply of the cone scale. Arnoldi's account (1900) is the only earlier one which gives anything specific on the internal morphology of the Big Tree. His studies were made from material found in cultivation in the old world and do not include the embryo. The accounts of Shaw (1896) and Lawson (1904) were confined to S. sempervirens. Looby and Doyle's investigation covering fertilization and the proembryo is the first and only investigation of the Big Tree which includes a detailed account of these stages.

In the following account of the gametophytes I am adding some new observations and repeating here and there a number of features covered by previous investigators. This duplication appears necessary in using my figures in the presentation.

Figure 1 is a drawing showing the upper surface of a cone scale just before pollination. At this time the ovules are still very small and immature, consisting only of a nucellus bordered by the integument, which is merely a narrow rim. They may be described as appearing erect at this stage. With the enlargement of the cone scale after pollination, the ovules grow rapidly and soon develop to the

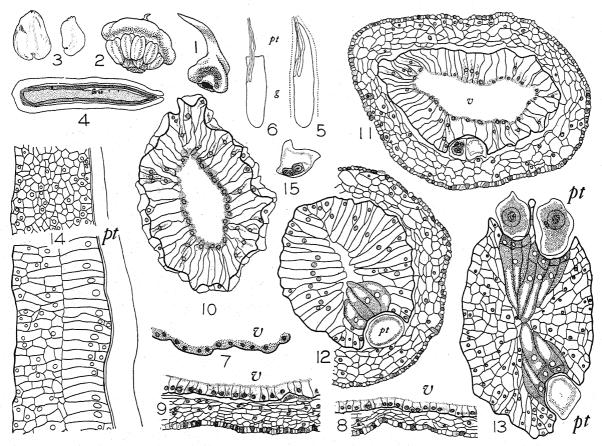


Fig. 1-15.—Fig. 1. Ovulate cone scale of Sequoia gigantea viewed from above, before pollination. Ovules are borne in two crescentic rows. X5.—Fig. 2. Ovulate cone scale of Sequoia gigantea from a cone two-thirds grown, showing two rows of ovules.—Fig. 3. Ovules showing winged margins and variations in shape—placed with micropyles pointing upward.—Fig. 4. Section of ovule showing micropyle at right and nucellus (shaded) containing female gametophyte beside pollen tube shortly before fertilization. ×6.—Fig. 5. Male and female gametophytes removed after fertilization showing lateral position of pollen tubes; dotted line = boundary of nucellus before removal. ×6.— Fig. 6. Same, with upper portion of female gametophyte removed by dissection.—Fig. 7. Part of megaspore membrane showing cytoplasm and nuclei surrounding large central vacuole (v) in free-nuclear stage of female gametophyte. July 14, 1936. ×100.—Fig. 8. Portion of wall of nucellus from transverse section through upper portion of female gametophyte showing beginning of wall formation between nuclei which are still lying close to megaspore membrane; v, position of large central vacuole.—Fig. 9. Same as fig. 8 through lower portion of same nucellus and female gametophyte. July 17, 1936. ×100.—Fig. 10. Transverse section through female gametophyte at level below ends of pollen tubes, showing later stage in wall formation, the nuclei and cytoplasmic layer having moved opposite the newest part of cell wall. July 17, 1936. ×100.—Fig. 11. Same as fig. 10 but including nucellus, through lower portion of pollen tubes. Two pollen tubes are shown between nucellus and megaspore membrane imbedded in a groove of female gametophyte. The pollen tube at the left shows body cell slightly enlarged beside stalk and tube nucleus; v, central vacuole. ×100.—Fig. 12. Slightly later stage than fig. 10 and 11, with cells meeting at center and closed, with their nuclei centrally placed in each cell. A group of archegonial initials are recognizable near the pollen tube (pt). July 17, 1936. ×100.—Fig. 13. Transverse section of gametophyte in later stage, July 26. The body cell of pollen tubes have enlarged greatly, and the archegonia have cut off primary neck cells. ×100.—Fig. 14. Two parts of longitudinal section of gametophytes showing condition between that of fig. 12 and 13. Many potential archegonial initials are shown on the side next to the pollen tube (pt). July 26, 1936.  $\times 100$ .—Fig. 15. Section of pollen tube showing slightly enlarged body cell beside stalk and tube nuclei.

size of the mature seeds. The micropyles are now directed toward the cone axis. As figure 1 shows, the ovules are attached to the cone scale in two crescentic rows, and the resulting double row of ovules is still evident on the scale of a cone two-thirds grown (fig. 2). Figure 3 shows two fully enlarged ovules placed with their micropyles pointing upward and indicates that some ovules and

seeds are deformed or curved in several directions by local pressure during development. Figure 4 shows a section through an ovule and illustrates the lateral position of the archegonia and pollen tube, an unusual condition for conifers. The pollen tube enters the nucellus and grows into the position shown in the figures (fig. 4–6 and 11–13) long before the female gametophyte has become cellular.

The archegonia are produced on that side of the female gametophyte which is in intimate contact with the pollen tube. Figures 5 and 6 show the condition, as found in dissections of material shortly before fertilization, for an ovule with a pollen tube situated on its flat side similar in section to figure 11. More frequently the pollen tubes pass down close to the edges of the female gametophyte, as shown in

figures 12 and 13.

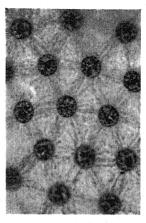
If the female gametophyte is removed from the ovule by dissection at a stage after fertilization, the pollen tubes will usually be found adhering to its surface and appear to be imbedded in a groove, as shown in figure 5. Of course the megaspore membrane separates the pollen tube from the surface of the female gametophyte. The pollen tube is firmly attached by the fertilization tube slightly above or near the mid-point of the female gametophyte so that the upper portion of the latter may be removed, as in figure 6, without disturbing the embryo. Both the megaspore membrane and pollen tube become very thin near the fertilization tube, and openings are formed through both at the time of the entrance of the male nuclei. The pollen tube is slightly enlarged at this place, and its end may extend downward for as much as 0.4 mm. below the fertilization tube, remaining somewhat smaller above and below this point of attachment. In some cases the fertilization tube is formed nearer the end of the pollen tube, giving a shorter "tail."

The earliest stages in the development of the pollen tube were not observed. Since the pollen grain is binucleate at shedding, there is only a single cell division which takes place before the stage shown in the transverse section of a tube, figure 15, where we have an enlarged body cell beside the stalk and tube nucleus. Both of the last named nuclei are without cell walls surrounding them. One of the pollen tubes in figure 11 is in very nearly the same stage, while figure 13 shows the body cell greatly enlarged about ten days prior to fertilization. Looby and Doyle report that they observed the division of the body cell and show that male gametes are formed as in Taxodiaceae and Cupressaceae. Evidently the gametes do not consist of

naked nuclei as found in pines.

As in other conifers, the early stages of the female gametophytes of Sequoia are free-nuclear. In early stages the nuclei are situated at the periphery very close to the megaspore membrane surrounding a very large central vacuole. In one of the ovules studied, the writer counted about 900 free nuclei. The oldest free-nuclear stage which could be found showed close to 4,000 free nuclei, while the stage in which walls have just begun to form (fig. 8, 9) had more than 6,000 nuclei, so that one may estimate 12 or 13 simultaneous divisions before walls begin to form in the female gametophyte. Mitotic stages were not observed during the free-nuclear stage, and there appeared to be occasional differences in the sizes of the nuclei of the last stages; therefore, there may be a ques-

tion as to whether or not all the divisions—that is, up to the last mitosis-occur simultaneously. Figure 16, a photomicrograph of the dissected surface of the megaspore membrane at the time of wall formation, indicates a very regular spacing between these nuclei and, with few exceptions (not shown), a great uniformity in their size.



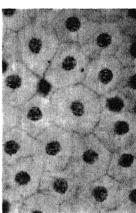


Fig. 16, A and B.-A (left). Surface view of megaspore membrane showing free nuclei spaced in pattern with phragmoplastic connections, before the appearance of cell walls.-B (right). Similar view after cell walls have begun to form.

Figure 7 shows a sectioned portion of the megaspore membrane with the adhering fringe of free nuclei in a late stage before walls are formed. At this stage the center of the gametophyte has a large clear vacuole (v). Wall formation begins at the periphery, as shown in figures 8 and 9. The former is a section through a place one-third of the distance from the tip of the nucellus and the latter about two-thirds this distance from it. Figure 16a is a photomicrograph of a dissected specimen just before walls appear. It shows a beautiful pattern of phragmoplastic connections developed between all neighboring nuclei. Figure 16b shows a subsequent stage after walls have become visible. This corresponds to the stage shown in figures 8 and 9. Figures 10 and 11, taken at different levels of the same gametophyte in slightly later stages (fig. 10 below the pollen tubes and fig. 11 through two pollen tubes), show that the nuclei, which are very close to the megaspore membranes, move inward as the walls are formed and tend to remain opposite the newest portions of the walls until the individual cells become closed; after this they become more centrally placed in each cell (fig. 12). It will be noted that many cells become crowded and are closed before the center is approached. Sometimes a central space remains for a time but becomes closed by elongation of the newly formed cells. Figure 14 shows a longitudinal section in which nearly all the cells touching a pollen tube (pt) have been delayed in their division in the region where archegonia appear.

Archegonial initials appear very early and are found in large numbers on the side of the megagametophytes adjacent to the pollen tubes. The initials are the first walled cells. Some of these may subdivide to form ordinary cells, others persist for a time, appearing as larger cells containing large nuclei in a dense cytoplasm. If they cut off smaller primary neck cells, as shown in figure 13, they have progressed another step in differentiation as archegonia, but some become partitioned into several or many smaller cells. The neck cell divides into two cells and finally forms a neck of four cells, as shown by Looby and Doyle (1937). Archegonia that become diverted to form ordinary gametophytic cells later may, in certain stages of becoming partitioned, give the appearance of enlarged jacket cells. The matured archegonia may appear in groups with only a cell wall dividing them, but there is no specially differentiated jacket that wholly surrounds such a group; therefore we do not find the type of archegonial complex observed in Cupressaceae.

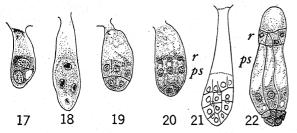


Fig. 17-22. Stages in the proembryo of Sequoia gigantea; r, rosette; ps, prosuspensor. After Looby and Doyle (1937). ×100.

Nearly all the irregularly scattered jacket cells derived from archegonial initials that have been recently partitioned disappear before fertilization. Very soon after fertilization, the unfertilized archegonia either collapse or become partitioned so that few traces of them may be found in gametophytes with embryos that are a week old. However, the pollen tube persists and may be found for many months afterward.

During the development of the female gametophyte, many nuclei may be observed in mitosis. The number of chromosomes counted during this haploid

stage is eleven.

The method of development shown by the female gametophyte (fig. 8–12) confirms Arnoldi's observations (1900) and adds other details. He described this method of "endosperm-formation" as alveolar and simultaneous throughout the female gametophyte in contrast to the method which he had previously observed in S. sempervirens (Arnoldi, 1899a) in which the two end regions of the gametophyte become differentiated from the central region. The free nuclei that are found in the end regions are spaced evenly throughout the gametophyte before walls are formed simultaneously between all nuclei, and only the central part forms the alveoli which develop centripitally around a

central vacuole. As shown in figures 7 to 14, the megaspore membrane is thick and very conspicuous. It is thickened at the basal end of the female gametophyte and becomes somewhat thinner as the micropyle is approached, is two-layered, and ranges in thickness from 2 to  $3\frac{1}{2}$  microns.

In S. sempervirens several investigators have reported the occurrence of numerous female gametophytes within the same ovule. It appears that these come from as many megaspores and compete with each other so that all but one or a few are eliminated. Naturally I sought for evidence of this type of development in S. gigantea, but no traces of a second female gametophyte were found even in the earlier free-nuclear stages. If this feature occurs at all, it takes place only in a very early stage of development.

Although by cutting more material I could add additional details concerning the earlier history of the development of the gametophytes and archegonia, my collections were not made frequently enough to close all the gaps in the series. Therefore, since Looby and Doyle have suggested that they still have some of these additional details under investigation, we may await with interest the

results of their researches.

THE EMBRYO.—For the stages which include fertilization and the proembryo the very full account of Looby and Doyle (1937) may be summarized. As mentioned previously, these investigators have demonstrated the important fact that in S. gigantea, there are several free-nuclear divisions in the early zygote as in Cunninghamia, Cryptomera, and other conifers. In respect to this feature, the Big Tree differs radically from its supposed close relative S. sempervirens, in which a cell wall is formed after the first division of the zygote. Figures 17 to 22 are successive stages taken from Looby and Doyle indicating that the first walls are formed after 8 free nuclei have been formed and that these nuclei and the cytoplasm of the egg at this stage occupy the lower half of the archegonium. The proembryo is organized into three or four tiers of cells. The lower tier at the stage of figure 20 is commonly 3-celled or 2-celled and the upper tier which divides again is 5-celled or 6-celled. Subsequent division may occur at both ends of a proembryo, forming an extra tier such as that shown in figure 21 and other exceptional cases, so that a group of rosette cells in one or two tiers is formed above the tier of 5 or 6 elongating cells; also the 2 or 3 embryonic cells situated below the elongating cells increase to form a group of at least 5 or 6 cells.

It appears that the cells of the rosette group may become enclosed by cell walls with an open space above, or they may proliferate to fill out more of the upper part of the archegonium. This may add irregular tiers of cells in the rosette. The central tier soon begins to elongate, thrusting the lower group of cells, as they increase in number, deeper into the tissue of the female gametophyte. Looby and Doyle did not follow the embryogeny beyond

the stage shown in figure 22, merely reporting that some type of polyembryony ensues.

Figure 23 shows an embryo of this early stage which is still associated with the pollen tube. Here all the cells of female gametophytes were omitted, but no rosette cells could be found. This embryo is smaller than most others of the same stage, and the terminal group of cells is still composed of only four cells. Any of the cells in these stages may collapse, which would account for some of the irregularities in cell number and arrangement.

As in my descriptions of certain other conifers, the elongating suspensor cells constitute a prosuspensor—a group of cells represented by a distinct tier of cells in the proembryo, elongating in unison and constituting a specially differentiated part of the entire suspensor system. Figure 24 shows two neighboring zygotes with their prosuspensors elongating. The one at the left has formed a large irregular group of rosette cells, crowded into the upper part of the archegonium; the one at the right, a smaller rosette with an open abortive space above. Four or five cells are found below in the terminal

embryonic group.

Figure 25 is a similar but larger and slightly older pair of embryo systems coming from larger archegonia. There are well developed rosette embryos and also some free nuclei above them in the remaining portion of one of the archegonia. The terminal group of cells has increased here to 8 or 9. A later stage is shown in figure 26. The prosuspensor has become long and twisted, and there are many more cells in the terminal group. This embryo system has only a few rosette cells, one of which has formed a rosette embryo. Thus we find considerable variation in the extent to which the rosette is developed. All the foregoing stages were found in collections of August 18, while the next stages were collected on September 8, an interval of more than two weeks. Figure 27 shows the smallest embryo complex which could be found in the collection of September 8. It shows traces of collapsed prosuspensors (ps) with more than a dozen individual embryos growing on their own primary suspensors (s), and some other peculiar embryos (probably developed from the rosette cells), pushed back against the crushed prosuspensor (ps). Evidently the individual cells visible at the tip of a prosuspensor such as that of figure 26 have each given rise to a terminal embryonic cell and a primary suspensor cell. The primary suspensor cells (s) have elongated greatly, while each of the embryonic cells has divided into two or three cells to form the complex mass of embryonic material of figure 27.

Figure 28 represents a stage collected nearly a month later. Only the lower portion (about half) of the greatly folded suspensor system is shown. The older parts have all collapsed, including the primary suspensors, portions of which are shown above at s in this figure. Embryonal tubes of the first, second, and third order  $(e_1, e_2, \text{ etc.})$  have ap-

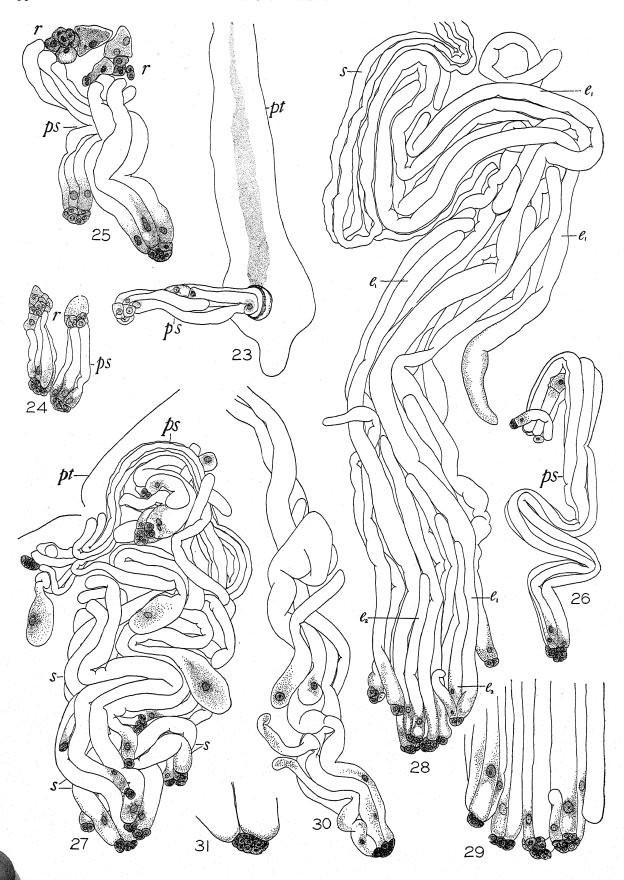
peared, so that now each embryonic group is borne on the end of a secondary suspensor composed of two or three embryonal tubes. The longest of the embryonal tubes are the oldest; the shorter ones may in many cases be traced throughout their length. Embryos may become aborted or dissociated at any stage.

Some of the smaller embryos shown here on a single suspensor segment probably originated from an unequal fractionation of larger embryos, a sort of secondary cleavage or "budding." For instance, the two embryos shown at the extreme left and right of the group in figure 28 are on suspensor segments which, as they are traced upward, do not reach all the way back to join even the primary suspensors (s), not to mention the prosuspensor, which would require more than twice this space for portrayal. These embryos were evidently derived from lateral lobes that were split away from the larger embryos and in breaking away included an elongating embryonal tube. The latter is now the single-celled suspensor segment to which they are attached. Then when the embryonic cells of these fractions abort, there may result a lone elongated cell.

Many embryonic cells were found isolated among the folds of collapsed primary suspensors, and there were some in the central and lower region of figure 28, which were not shown because they were covered by the mass of embryonal tubes,  $e_1$ ,  $e_2$ , etc. Furthermore, in these and later stages there were often many very small embryos which grew out of the gametophyte to become imbedded in the nucellus at the level of the fertilization tube. These were probably derived from rosette embryos. Small embryonic fractions, regardless of their origin, gradually become aborted, so that the number of sizable embryos remaining in the terminal group becomes smaller and smaller with time.

Figure 29 shows a stage similar to that of figure 28, with the terminal embryos somewhat older. Figure 30 is a single embryo and its secondary suspensor of the oldest stage collected October 15. Usually the embryos do not become much larger than this one, passing the winter in this or a similar stage. Figure 31 shows the tip of an embryo which was dissected from the softened ovule of a herbarium specimen collected by Mr. W. T. Frost on November 22, 1934 (University of California Herbarium).

The early embryo of S. gigantea appears to grow by means of an apical initial cell. The apical cell is not as conspicuous as in some other conifers and persists only long enough to form a half dozen segments or even fewer. The primary embryo divides into a primary suspensor cell and an embryonic cell; this embryonic cell may be considered as a hemispherical apical cell and the primary suspensor its first segment, which elongates and pushes the embryonic cell forward. Meanwhile the apical (embryonic) cell divides by a vertically placed wall; one of the daughter cells is the segment, the other is the apical cell. The segment soon elon-



gates, becoming an embryonal tube  $(e_1)$ , and the apical cell divides again and again cutting off segments on several sides. These segments may divide and elongate wholly or in part as embryonal tubes  $(e_2, e_3, \text{etc.})$ . The segments are not easily recognizable because some become elongated embryonal tubes. The apical initial usually persists at least to the end of the growing season when growth is checked by winter.

The next stages observed were before and at the time of formation of cotyledons. I am not including figures, since I observed only about a dozen healthy ovules in these stages. The cotyledons are formed late in July of the year following fertilization. A stem tip primordium is recognizable at the tip of a cylindrical embryo of several hundred cells; which is soon surrounded by a circle of about four cotyledon primordia. The embryo develops rapidly during August and September of the second year, so that the seeds are morphologically mature at the end of the second season following fertilization. In spite of the extensive cleavage polyembryony, nearly every seed has only one surviving embryo.

That the Big Tree is polycotyledonous may be observed in seedlings. The average cotyledon number in 73 seedlings was found to be 3.7. Five had 5 cotyledons, 40 had 4 cotyledons, 28 had 3 cotyledons. None of the 73 had fewer than 3 cotyledons.

The position of the archegonia is near the midpoint of the female gametophyte, but the latter continues to grow considerably throughout its lower half, thus crowding the upper portion. This belated growth includes an elongation of the nucellus and also an increase in its diameter. These structures are crowded upward into the space between the integuments. In the older stages collected in October, the upper part of the wall of the nucellus is usually so crowded that it has become folded transversely and wrinkled, and by the time the seed matures one would scarcely suppose from the position

of the embryo that fertilization took place so near the middle of the gametophyte.

From the drawings and magnifications given, it may be noted that the embryos of S. gigantea are very small. Whether we take one of the embryonic cells of figure 23 or one of those in figure 27, there is not a great variation in cell size. We may consider either of these embryos still essentially onecelled, since the cells in excess of one will all go to form suspensor-like embryonal tubes. If we consider the one-celled stage from figure 27, where it may be seen in the center of the lower group of primary suspensors (midway between the two lower S markers), and estimate its volume, we find this to be about 20,000 cubic microns. Thus in the Big Tree which may eventually reach a volume of 1,640 cubic meters (estimated for the General Grant Tree), the organism enlarges  $82 \times 10^{15}$ -fold.

It is difficult to comprehend the magnitude of this figure, so that it may be interesting to make a comparison with objects of appreciable size. I have made a computation using the size of the earth for comparison and found that the mature specimen of S. gigantea is as much larger than its embryo as the earth is larger than a building similar in size to the dome of the Adler Planetarium.

Discussion.—The development of the female gametophyte of the Big Tree follows the usual program found in other conifers. A long free-nuclear stage involving a succession of 12 or 13 divisions is found in its development, and the archegonia are laterally placed. These prothallia develop singly rather than in competition with other female gametophytes as in the redwood.

The megaspore membrane, which is two-layered and very distinct in *S. gigantea*, is not so thick and well developed as in Pinaceae, nor so thick as in certain other conifers such as some of the podocarps.

The writer has reported that the potential archegonial initials are very numerous, involving nearly all the cells in certain regions bordering on

Fig. 23-31.—Fig. 23. Early embryo system (dissected) still associated with pollen tube (pt); ps, prosuspensor. August 18, 1936. ×100.—Fig. 24. Two neighboring zygotes probably resulting from fertilization of neighboring archegonia by same pollen tube; r, rosette cells; ps, prosuspensors bearing a group of embryonic cells below. August 18, 1936. X100.—Fig. 25. Two similar zygotes in slightly later stage showing twisting prosuspensors (ps) bearing an increased number of cells in the embryonic group below. The rosette groups (r) above have divided with free nuclei still showing in upper portions of archegonia. August 18, 1936. ×100.-Fig. 26. Older stage with prosuspensor (ps) considerably elongated, about 10-12 embryonic cells below and only a few rosette cells or embryos above. August 18, 1936. ×100.-Fig. 27. Much older stage of development than fig. 26 after cleavage of the group of embryonic cells shown in fig. 26. Each embryo is found borne on its own greatly elongated primary suspensor (s) which has pushed back the collapsed prosuspensor (ps) shown above. Rosette embryos may be seen scattered about in the upper portion of this figure (pt, edge of a pollen tube). September 8, 1936. ×100.—Fig. 28. Lower portion of an embryo complex after the primary suspensors (s) have begun to collapse. Each embryo unit is now borne on one or more embryonal tubes, whose order of origin and elongation  $(e_1, e_2, \text{ etc.})$ are indicated by their lengths. At this stage it is very difficult to identify the completely collapsed prosuspensors, and only some of the primary suspensors could be traced. Many small embryos, probably rosette embryos, are scattered about in the collapsed upper portion, omitted from the drawing. October 5. ×100.-Fig. 29. Tip of an embryo complex similar to fig. 28. October 11.-Fig. 30. One of the oldest embryos obtained on October 15 singled out to show the succession of embryonal tubes from the secondary suspensor. The upper portion of the suspensor probably represents  $e_1$  and  $e_2$ . There are approximately 12-15 cells in the embryo below. This is approximately the condition found at the approach of winter. October 15. ×100.-Fig. 31. Tip of an embryo slightly older than fig. 30, taken (with permission of curator) from a dried specimen found in the University of California Herbarium collected in Sequoia Park by W. J. Frost on November 22, 1934. X100.

the pollen tubes. Of course it is possible that they are actually not so numerous and that I have been unable to distinguish such initials morphologically from other large cells in that position. However, it must be remembered that very many of these divide unequally to cut off primary neck cells and larger central cells, thus closely resembling the archegonia of other conifers at this stage. Many of these develop no farther and finally subdivide into ordinary gametophytic cells; in a smaller number the primary neck cell divides into two neck cells; a few into four neck cells, as Looby and Doyle (1937) have shown. The work of these authors does not show that a ventral canal cell or nucleus is formed. I have found none, but the archegonia in my own material may have been too immature to include this stage if it is present. Ventral canal nuclei have been reported in related genera so that one would expect to find them in Sequoia, but thus far their occurrence has not been demonstrated.

The embryogeny of S. gigantea resembles, in general, that of Sciadopitys (Buchholz, 1931) more nearly than that of any other conifer. In Sciadopitys there is at least one more free nuclear division; Lawson (1910) shows a section through a proembryo with six of the eight free nuclei, so that one may judge that walls may form after the 8 free nuclei have divided to give rise to 16. The archegonia are very much larger in Sciadopitys, terminal in position, and not grouped into an archegonial complex. Another difference is that the embryo of Sciadopitys is usually dicotyledonous, but has three cotyledons occasionally, while that of S. gigantea is tetracotyledonous.

Aside from these important differences, the similarities in embryogeny between the Big Tree and Sciadopitys are very striking. Both form a prosuspensor, which is somewhat ephemeral in the Big Tree; both have rosette embryos, not constantly present in Sciadopitys and relatively better developed in S. gigantea; both have a similarity in the formation of the primary embryos from the initials borne on the end of the prosuspensors. The details involving the formation of primary suspensors, and the apical cell growth in each embryonic unit are similar.

The embryos, borne on primary suspensors, appear different only as to relative proportions. The primary suspensors and embryonal tubes in Sequoia are more slender than in Sciadopitys, and there appear to be a larger number of successive additions of embryonal tubes.

When the embryogeny as given here is compared to that of the redwood, S. sempervirens, as pieced together from the accounts of Shaw (1896), Arnoldi (1900), Lawson (1904), and others, many important differences are discovered. These differences will be discussed more fully in a subsequent paper in which the writer has included his own investigations on the redwood. It will be shown that the embryo of S. sempervirens, aside from having no free nuclear stage in the proembryo, omits the

prosuspensor entirely. There are also many other features in which the two species of *Sequoia* differ; in fact, it may be shown that the two species are so far apart in respect to these morphological criteria that they should be regarded as belonging to separate genera.

### SUMMARY

The general morphology of the ovule of S. gigantea, its relation to the cone scale, and the position of the gametophytes in the ovules of a cone which is nearly full grown are briefly described.

The development of the female gametophyte is described with reference to the extent of the free nuclear stage, followed by an alveolar type of centripetal development, the origin of archegonial initials, and their relation to the position of the male gametophyte. Archegonia have their origin in the first walled cells that are formed in a position adjacent to the pollen tubes; very many potential initials are found developing only to the two-celled stage, though relatively few develop fully into matured archegonia.

Archegonia are usually grouped into an archegonial complex or into several such groups, which differ from those of the Cupressaceae in the absence of jackets.

The mitotic figures observed in the female gametophyte permitted the counting of 11 chromosomes as the haploid number in S. gigantea.

The male gametophytes were observed with the usual large body cell, a stalk, and a tube nucleus. They form a fertilization tube which connects with the pair of archegonia that are fertilized.

The megaspore membrane is composed of two layers and is of considerable thickness—between 2 and  $3\frac{1}{2}$  microns.

As Looby and Doyle have shown, the embryo begins its development with free nuclear divisions; 8 nuclei are present when walls are formed. The proembryo fills the lower half of the egg and may eventually fill the entire archegonium. It is organized into tiers, a rosette tier or region which may include several tiers, a tier forming a prosuspensor, and a group of two or three cells below the prosuspensor which, by their further division, give rise to embryo initials.

As it elongates, the prosuspensor pushes the terminal group of embryonic cells forward, while the latter multiply to become a group of 8 or more embryo initials. The embryo initials cut off primary suspensor cells which elongate rapidly, soon pushing back and crushing the prosuspensor. The terminal embryo units divide by apical cell growth to form successive segments, the oldest of which forms the primary suspensor, followed by a succession of later-elongating embryonal tubes. Some segments borne on embryonal tubes may become dissociated and develop independently. Cleavage polyembryony is therefore very extensive, resulting in a large number of embryos per zygote.

As the end of the first growing season is reached, the embryos are still composed of only a dozen or more cells, entirely undifferentiated except for the polarity shown in the embryo-suspensor axis.

The number of surviving embryos decreases; finally during the following spring the successful embryo becomes larger, develops into a cylindrical mass, and forms about four cotyledons in July-August of the second year, thus reaching maturity only by the end of the second season of its development.

In general, the embryogeny resembles that of Sciadopitys more nearly than that of any other conifer thus far investigated and appears to differ in many important features from the embryogeny of S. sempervirens.

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# A NEW METHOD FOR THE CONSTRUCTION OF THREE-DIMENSIONAL CELL MODELS

#### James W. Marvin and Edwin B. Matzke

In a study of the shape of pith cells, a new method has been developed by which accurate three-dimensional models of individual cells may be constructed. The procedure consists essentially of making casts of hard wax of the cells, separating these casts, drawing each face of a cast by means of a camera lucida on bristol board, and then reconstructing the cell on a large scale by fitting the pieces of bristol board together.

Apparently the only technique thus far used in the careful study of the shapes of cells in tissues is Born's wax-plate method, utilized with marked success by Lewis (1923). This involves cutting the cells into serial sections, modeling each section in wax, and then rebuilding the cells in three dimensions by stacking the wax plates in sequence. The method to be described deals with the cast of the entire cell, rather than with numerous serial sections of a single cell.

The pith is first removed from a portion of an internode of a stem by carefully cutting away most of the external tissues. A strip of these tougher outer cells is left to afford rigidity. The section of pith is then immersed in absolute ethyl alcohol and placed in a partial vacuum chamber to aid in the removal of air. After the tissue is completely

<sup>1</sup> Received for publication January 11, 1939.

saturated with alcohol, it is transferred through three alcohol-xylol mixtures to xylol. The first mixture contains two parts of alcohol to one part xylol; the second contains equal parts of alcohol and xylol; the third consists of one part alcohol to two parts xylol. After one to two hours in each mixture, the material is transferred to pure xylol, in which it remains for twenty-four hours, with several changes.

The next step is impregnation with purified Montan wax, which is a lignite derivative with a melting point of about 85°C.<sup>2</sup> It is hard but not brittle at room temperature. This wax is soluble in warm xylol, and impregnation is accomplished by pouring a small amount of the melted wax into a vial of xylol containing a section of the tissue and placing the vial in an oven. After a few hours, more wax is added. Later, the xylol and wax mixture is poured off, and pure wax is substituted. This is changed once a day for three days, after which the material is removed from the vial of melted wax and placed in ice water.

The wax on the surface of the mass of cells is then removed with a razor, and the material is im-<sup>2</sup> This is one of a number of possible waxes kindly

suggested by Mr. John Leutritz, Jr., to whom grateful acknowledgment is here made.

mersed in cuprammonia. This is made by covering copper filings with ammonium hydroxide (Stevens, 1924). The material remains in this solution for a time sufficient to allow the cell walls to become mucilaginous. It is then thoroughly washed in water and is ready for examination. According to Farr (1938), the cotton fiber is transformed by cuprammonium hydroxide solution into a swollen viscous mass of cementing material containing the cellulose particles.

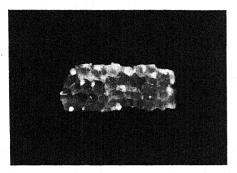


Fig. 1. Wax casts of a group of cells from the pith of Eupatorium purpureum L.  $\times 30$ .

Under a high power binocular dissecting microscope the individual cells are easily distinguished. They appear as shown in figure 1. The lumen of each cell is filled with wax which is stained a pale blue by the cuprammonia. With a pair of fine needles the wax casts of the cells can be separated from each other, each wax cast still surrounded by its cell wall. When dry, the cell wall is easily seen. The mucilaginous character of the cell wall when wet makes it possible to attach the cast of the cell to the tip of a fine glass bristle or thread.

The glass bristle, which is "L" shaped, is attached to the manipulator shown in figure 2. It appears as a thin black thread-like line in the illus-

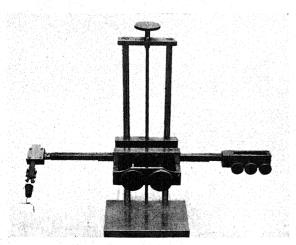


Fig. 2. Manipulator used in orienting individual cells under binocular dissecting microscope. Description in the text.  $\times \frac{1}{16}$ .

tration and is mounted so that turning the short arm of the "L" revolves the bristle on its long axis, to the tip of which the cell adheres. The long arm of the glass thread is oriented perpendicularly to the rod to which it is fastened. The rod is thrust into a cork which is held by wire forceps. The cork and rod can be rotated on their long axes by a geared adjustment. With these two movements the cell can be moved in two planes. In addition, the entire arm on the left, including the bristle, cork, and forceps, is in turn attached at right angles to a second heavier horizontal rod which can be turned by gears, moving the bristle up and down in an arc. The heavy horizontal rod and consequently the glass thread can also be moved from right to left by a rack and pinion, as well as forward and backward and up and down by two other adjustments.

With this device it is possible to orient each face of the cell in sequence in the horizontal field of a high power binocular dissecting microscope. The outline of each face, thus oriented, is drawn with a camera lucida on bristol board. Then the faces are cut out and glued together in proper order to make a three-dimensional model of the cell, in a fashion similar to that used in modeling the orthic tetrakaidecahedron (Matzke, 1931).

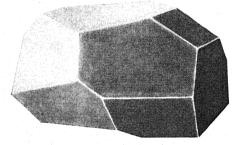


Fig. 3. Natural size drawing of a model of an unusually large pith cell of *Eupatorium purpureum* L. The original pith cell is magnified 170 diameters in the model.

From such a model the number of faces may be determined and also the number of edges per face and the number of dihedral, trihedral, and tetrahedal angles. In addition, it is possible to compute the surface area of the faces as well as the volume of the cells. A comparison of the cells of the original pith tissue with the wax casts indicates that the technique used causes no appreciable distortions and no changes of major significance in shape.

A drawing of a model of a pith cell of Eupatorium purpureum L. is shown in figure 3. The pith cell is magnified 170 diameters in the model, and the latter is drawn natural size. This matches the original cell, face for face. The number of contacts, fifteen, of which seven appear in the drawing, as well as the kind of contact—whether triangular, quadrilateral, pentagonal, etc.—can readily be determined, and also the number of dihedral and trihedral

angles. The curvatures of some of the faces and edges are not reproduced in the model and are not shown in the drawing. This particular cell is an unusually large one, approximately twice the average length of the cells shown in figure 1.

Using the technique described above, studies of the pith of *Eupatorium purpureum* L. have been made, and similar work on other genera is in prog-

ress.

#### SUMMARY

A new technique is described for modeling cells in three dimensions, to be used in studying their shapes. The cells are impregnated with hard wax, the casts are separated, each face is drawn with a camera lucida on bristol board, and the pieces of bristol board are then fitted together in proper

order to build a scale model of the cell, much enlarged.

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### THE CONCEPT OF SPECIES BASED ON EXPERIMENT 1

Jens Clausen, David D. Keck, and William M. Hiesey

The Linnean species concept was based upon the supposition that species are separate entities unable to cross or at least unable to produce very fertile hybrids. In his mature years, Linnaeus found by experiment that some species could produce partially fertile hybrids (1790), and he arrived at a concept of evolution of species by hybridization (Systema Vegetabilium, 1774). Unfortunately, Linnaeus' taxonomic followers did not heed his advice on experimental work, nor were they very much influenced by Koelreuter's (1761–1766) work on interspecific hybrids. Consequently, the early taxonomists emphasized the concept of the rigidity of species.

Jordan (1846) proved by experiment that races of one Linnean species from different environments remained distinct when grown in a standard garden. This discovery also left very little influence, because, in accordance with the prevailing beliefs, Jordan interpreted this to mean that each race was a distinct species. To taxonomists this appeared absurd, and they discredited the new experimental trend. Furthermore, Darwin's books diverted the energies of taxonomists from the experimental to a more philosophical approach, which culminated in the development of a "phylogenetic" system based on comparative observation alone.

As modern genetics developed in this century, it was rediscovered that hybridity is not a rare phenomenon and that many hybrids between species are partially fertile. This, together with the evolutionary philosophy, led to the extreme viewpoint that species after all are but abstractions of the human mind. This trend of thought, widespread among geneticists and physiologists, was nourished by the activities of a contemporary, non-experimental school of splitters among taxonomists.

<sup>1</sup> Received for publication December 16, 1938.

It is evident that the species problem is too complex to be solved by herbarium work alone and that the geographical and ecological methods, indispensable in all good descriptive taxonomy, are also insufficient. Cytological and genetical methods of themselves are just as limited, for species and other evolutionary units are closely connected with a natural environment. Very few cytologists or geneticists study their plants or animals except in the laboratory, and the forms studied are seldom representative of those found in any natural environment.

Moreover, in most genetic experiments performed with plants from natural environments, there are two facts concerning truly interspecific hybrids which have not been duly emphasized. One is that only a small percentage of the genic recombinations survive, the other that vigor of the survivors is reduced, so that only few members of the second generation are fit for the severe competition in nature. There actually is a natural reason for the fact that although crossings constantly take place, the species have remained remarkably the same for centuries, as evidenced by herbarium specimens, and even for ages, as shown by geological records.

The writers have performed extensive experiments on several plant groups during a series of years, viewing the problems from morphologic, geographic, ecologic, cytologic, genetic and to some extent also physiologic angles. The largest body of evidence has been obtained from the Madiinae, a West American subtribe of Compositae. Out of some 400 hybrid combinations attempted, involving seventy species of six genera, about 200 succeeded, and the others were non-viable or complete failures. Similar experiments were done in a group of three species of Zauschneria (Onagraceae), in Potentilla

(Rosaceae), Viola, and various other genera. Many illuminating data have been obtained from a study of the reactions of many plant species transplanted to different climates. Also, the literature on interspecific hybridizations from the last two decades abounds with evidence on the nature of species, although the taxonomically important information has not always been emphasized.

Here is not the place to enter into a detailed discussion on the nature, composition and evolution of natural species. This is the subject of volumes in preparation based on our detailed experiments. It is sufficient here to state that plants are organized into groups, the members of each of which are able to interchange their genes freely in all proportions without detriment to the offspring. Such groups are separated from one another by internal barriers that are of a genetic-physiologic nature (including chromosomal barriers) that prevent such free interchange. These natural groups correspond fairly closely to the species of the moderately conservative taxonomists working with plants that reproduce sexually.

This criterion for species, now substantiated by experiment, is the same that Turesson (1922) previously applied to the ecospecies. Consequently, we use his terminology to distinguish species whose status has been determined by experiment. The ecospecies becomes the experimental homologue of the taxonomic species. Also Dobzhansky (1937) has recently called attention to the importance of the internal ("physiologic") barriers separating species, noting that commonly they coincide with the delimitations of the species as accepted by systematists.

For simplicity, the experimental concept of species has been put in tabular form.<sup>2</sup>

Admittedly it is impossible to produce definitions that will cover all differences between systematic units, because species are in all stages of evolution. Nevertheless, this classification is based upon criteria of superior importance for the continuation of

<sup>2</sup> The principles for this classification were presented in a symposium before the American Association for the Advancement of Science in St. Louis, January, 1936, and also elsewhere (see literature references).

the species in nature. Also, it provides a scale permitting us to evaluate natural units in transition between ecotype, ecospecies, and cenospecies. It limits subjective judgment to a fair minimum, making possible a comparison of various types of species characteristic for different parts of the world.

The degree of separation used in this system of classification is based upon two kinds of barriers internal (hereditary) and external (environmental). The internal barriers are possibly all geneticphysiologic, expressed through incompatibility and intersterility or through weakness of the hybrid offspring. Ultimately, it is probably the genes that govern the rates and inception of the metabolic processes that are so evidently off-balance in offspring of interspecific hybrids. The external barriers are either ecologic or geographic. A distinct geographic barrier is likely also to be ecologic. The most permanent barriers are the internal, because they persist even though the environment changes in a changing world. They are therefore used in distinguishing species. A qualifying statement as to the internal barrier is necessary. Two forms may prove intersterile by direct test, although they interlink through one or several intermediaries that are interfertile with both. The barrier separating such forms is not considered specific, because genes may be exchanged through the intermediary.

Cenospecies.—If the genetic barrier is absolute, it separates groups so perfectly that they are able to exist together in one environment without intermixing. This kind of barrier is characteristic for individuals belonging to different cenospecies or species complexes. The cenospecies are groups of major evolutionary importance, comparable to but not at all identical with the taxonomic section, and may contain from one to many ecospecies. Members of different cenospecies may cross, but the hybrids are sterile unless doubling of chromosomes (amphidiploidy) takes place, in which circumstance a new species has evolved—a new step in the evolutionary process.

Ecospecies.—If the genetic isolation is only partial, we usually find that there is an additional geographic or ecologic isolation. This kind of separa-

TABLE 1. The concept of species.a

Degree of separation  Internal  External	Hybrids fertile, second generation vigorous	Hybrids partially sterile, second generation weak	Hybrids sterile or none
In different environments	Distinct subspecies ECOTYPES	Distinct species ECOSPECIES	Distinct
In the same environment	Local variations of one species BIOTYPES	Species overlapping in common territory (with hybrid swarms)	species complexes,  CENOSPECIES

<sup>&</sup>lt;sup>a</sup> The systematic units based on experimental evidence are in capitals, their homologues based on external characteristics are in italics.

tion, partly genetic and partly ecologic, is characteristic of ecospecies of one cenospecies. The ecospecies correspond to but are not always identical with the taxonomic species. To a limited extent, ecospecies of one cenospecies can exchange genes across the barriers, and they may show parallel variation on account of this exchange through the ages. If crossed, their hybrids are partially sterile, whereby non-viable sex cells are eliminated, or the second generation offspring is weak; most often both these conditions obtain. Weakness of the second generation may manifest itself in various ways: many individuals are slow-growing dwarfs, or subnormals; others are very susceptible to diseases to which the grandparents and even F<sub>1</sub> are immune; others again may be structural or anatomical misfits, to mention some examples. Any one or all of these weaknesses may characterize a hybrid population. In a world with strong competition such misfits have few chances of survival. A small percentage of the total offspring may be vigorous and fertile but tends to be eventually absorbed into one or the other of the parental species.

A group of ecospecies belonging to one cenospecies is found to inhabit a series of geographically or ecologically separated areas: one may inhabit a coastal region, another an inland region; one forested, the other open areas; one basic or alkaline, the other acid soils; one low, the other high altitudes; one a southern, the other a northern territory, and so on. In extreme cases, as in Salix or Viola, there may be as many as twenty or thirty ecospecies in one cenospecies. The genetic barriers between ecospecies are often produced by differences in number of chromosomes. Hybrid swarms may be found in nature where two ecospecies of one cenospecies meet or overlap.

ECOTYPE.—If there is no genetic but only geographic or ecologic isolation, the units are considered ecotypes of one ecospecies. They parallel, but are not nearly always identical with, the geographic subspecies. Ecotypes of one ecospecies may occupy a series of different habitats similar to that mentioned under ecospecies. The only difference between the two units is the lack of a genetic barrier between ecotypes. Like ecospecies, they differ by many genes. They hybridize freely where they meet, but at a distance from the point of contact they are quite pure. Ecotypes are evidently the forerunners of ecospecies, and there is no absolute gap between the two. Only a few cases, however, are truly intermediate and difficult to classify, and they are but a small fraction of the doubtful cases that arise in classification based exclusively on external appearance. They are inevitable in a dynamic, changing world.

LOCAL VARIATIONS. — Taxonomic classification stops with ecotypes. Within an ecotype or a subspecies, variation may be found of the kind that characterize different individuals of one population, or of a series of populations, in one kind of an environment. Neither external nor internal bar-

riers separate them. They hybridize and recombine thoroughly; millions of different combinations of these variations are possible in almost any species. They are the biotypes of genetic terminology, and it is obviously impossible to give taxonomic recognition to all these variants. Evidently they have very little selective value, otherwise they would not appear at random. In morphologic appearance some of these variations are very spectacular and have previously received wide taxonomic recognition, but we consider them only local variations not intended for inclusion in any taxonomic system.

Principles and application.—Two factors that are of great importance for living things are taken into consideration in this classification: the environment in which the organism lives, and the heredity, which must fit in the environment. Differences in form, long taken as the sole basis in classification, are now understood as important only as they mark genetic differences of various sorts, including those which have proved of selective value when subjected to the environment.

Ecospecies, ecotypes, and local variations are all based upon hereditary differences, but only the former two show any correlation to the environment. Differences in heredity cannot be seen directly but must be established by experiment. The simplest experiment of this kind is to grow samples of natural populations in a uniform garden. This eliminates the gross environmental differences and makes it possible to compare the heredity. The analysis of the heredity, however, can be undertaken only by hybridization experiments.

The morphological characters which taxonomists use in their classification have often been discredited as being of no importance in the life of the plant. This may be correct if we consider the characters by themselves, but our experiments show that they may be closely correlated to the physiologichereditary complexes on which ecotypes are based, such as earliness and capacity for survival in certain environments. Also, there is often an absolute correlation between morphological characteristics and the chromosome number or other internal barriers that separate species. When experiments have established the correlations, such character complexes become important indicators or key characters that can be used in tracing the distribution of ecotypes and ecospecies in the field and in mapping them with the aid of herbaria.

In asexually reproducing organisms, the present species criterion cannot be directly applied, although even among facultatively apomictic plants the number of successful hybrid combinations is very limited as found from experiments performed where crossings are possible. In other words, favorable combinations are selected for survival in these plants as well as in purely sexual species. A greater number of successful combinations are possible among apomicts than among sexuals because their F<sub>1</sub> hybrids are multiplied asexually and circumvent the exacting test of sexual reproduction

with its recombination of the parental genes in all proportions. Information on chromosome numbers and distribution of sexual progenitors and apomicts will often aid in understanding the evolution of and

the species problem in these plants.

TERMINOLOGY.—Botanists have agreed upon an international terminology in nomenclature, to which all should adhere. Nomenclatorially, each ecospecies of a cenospecies and also the monotypic cenospecies that contain only one ecospecies become taxonomic species. The ecospecies is therefore the basic taxonomic unit in experimental work. This will not greatly change the historic species concept, as mentioned, except in territories where the flora is insufficiently known. For practical reasons, two or more ecospecies that have no morphologic characters to distinguish them may be treated as one taxonomic species. The statement should be made, however, that biologically this complex contains more than one ecospecies, because genetic differentiation has preceded the morphologic.

The next unit permitted in nomenclature under the species is the subspecies. It is therefore natural to use this unit as the taxonomic homologue of the ecotype. Moreover, the term subspecies is commonly used for units of geographic or ecologic importance. It is less committed than the term variety, which was originally employed by Linnaeus to cover what we now would call environmental modifications and is commonly used also for garden forms. As in ecospecies, it is sometimes necessary to include more than one ecotype in a subspecies, stating that it consists of certain ecotypes that appear ecologically important but are morphologically indistinguishable. The objective is to have limits of subspecies (a morphologic term) correspond to the limits of one or a group of several ecotypes (an experimental term).

Whenever experimental evidence is lacking, the terms ecotype, ecospecies and cenospecies should never be used but only their homologues: subspecies, species, and species complex. If taxonomists are unable to use the experimental approach, they may nevertheless follow the geographic and ecologic principle in their work. In so doing, they will lay a solid groundwork for coming experimental investigations. They will make additional progress if they are able to determine chromosome numbers of several populations of their systematic units. If these differ, an internal barrier has almost invariably been set up, which would justify treating the units as distinct species, provided the morphologic and geographic evidence warrants it. first and simplest step in an experimental investigation is the cultivation of races from different environments in a standard garden, as Jordan (1846) did more than ninety years ago. This, however, can never replace the powerful analytical tool, systematic hybridization.

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## AUTONOMIC CYCLE OF RATE OF EXUDATION OF PLANTS 1

## Karl A. Grossenbacher

SINCE HOFMEISTER reported diurnal fluctuation in root activity in 1862, several investigators have substantiated his findings and attempted to obtain conclusive evidence as to whether or not such fluctuation represented a truly autonomic cycle. Studies in this laboratory have confirmed earlier work and, it is believed, have proved beyond doubt the autonomic nature of cycles in exudation.

White (1938) has recently reported that root pressure may reach very high values (greater than 6 atmospheres) and that there was a marked diurnal cycle in exudation of excised tomato roots. Unfortunately, he did not control light or temperature, so that it is not clearly demonstrated what the cycle he observed is related to.

In work reported elsewhere (Grossenbacher, 1938; Skoog, Broyer and Grossenbacher, 1938) in which considerable care was taken to control conditions during various tests of exudation and root pressure, results were obtained which indicated the existence of autonomic cycles. Complete proof of the autonomic nature of such cycles, however, must be dependent on obtaining control of the cycles. Otherwise, it is always possible to argue that changes in external conditions or some uncontrolled factor may be responsible for periodicity.

In order to study the nature of such cycles, plants were grown in the greenhouse and in cabinets (similar to those described by Davis and Hoagland, 1928) in which temperature, air flow, humidity, and light were under control.

Environments used.—(1) Greenhouse, "normal day"—without control; (2) cabinet A, "continuous day"—temperature 20°C.; relative humidity 70 per cent; constant air flow which, however, ranged from 0 to 100 feet/m. within various portions of the cabinet; light 100 to 300 ft. candles (12–300 watt lamps); (3) cabinet B, "inverted day"—identical with A, except that lights were off from 6 a.m. to 6 p.m.; (4) room C, "continuous night"—temperature 25°C.; relative humidity 90 per cent, gentle air circulation; and constant ruby light of low intensity; (5) temperature baths—continuous light (10 ft. candles) and temperature of 15° and 30°C.

Plants were grown in the greenhouse and in each of the two cabinets according to the usual solution culture technique. Each set represented 120 plants in 4 liters of half-strength Hoagland's solution (KH<sub>2</sub>PO<sub>4</sub>, 0.001 M; MgSO<sub>4</sub>, 0.002 M; KNO<sub>3</sub>, 0.005 M; Ca(NO<sub>3</sub>)<sub>2</sub>, 0.005 M). Iron and minor elements were added as needed. Seeds of Helianthus annuus (Russian Mammoth Sunflower) were planted in dry sand, watered, and placed in the cabinets or greenhouse. Seedlings were transplanted into solution after one week. Exudation

<sup>1</sup> Received for publication July 27, 1938. I wish to thank Prof. D. R. Hoagland and others in the Division of Plant Nutrition for their help and friendly criticism. tests were made with plants one to four weeks old in one or the other constant environments. (Cultures were continuously aerated during the test period.) The plants were cut off below the cotyledons, and short lengths of glass or rubber tubes were snugly fitted to the stumps (fig. 1). Exudates were

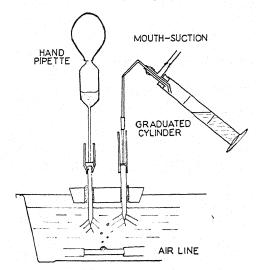


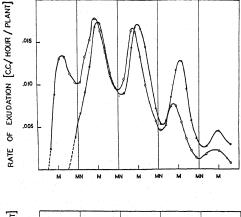
Fig. 1. Diagram illustrating methods of collecting exudates.

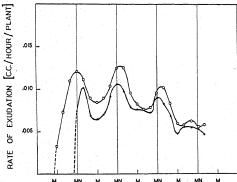
collected from these cups at three or four hour intervals for several days. Results are presented graphically in terms of rate of exudation (cc./hr./plant).

EFFECT OF LIGHT.—Results for the "normal day" plants are illustrated in figure 2. There was a pronounced cycle with maxima occurring during the day and minima during the night. Further, starting the tests one-half cycle apart caused no pronounced change in the time of their appearance or in rates of exudation. Similar results have been obtained in numerous experiments with normal greenhouse plants.

Plants raised under artificial conditions, with light during the night only, also showed twenty-four hour cycles (fig. 3). In this case, however, the maxima occurred during the artificial day and the minima during the artificial night (i.e., inverted day caused the cycle to be inverted).

It might be anticipated that there would be no cycle for plants raised under constant light conditions. Romell (1918), however, demonstrated that plants raised under continuous light did show a cycle. In his work this cycle coincided with that of plants grown under normal conditions. Here, however, five separate tests gave cycles which corresponded to those of inverted day plants. It was not until tests were started at different times of day that a possible explanation appeared. In figure 4





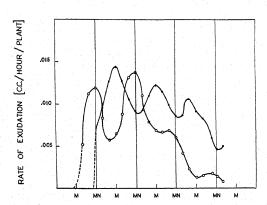


Fig. 2-4. Rates of exudation of plants decapitated at different times as indicated by dotted lines.—Fig. 2 (above). Plants grown in the green house.—Fig. 3 (center). Plants grown under condition of "inverted day."—Fig. 4 (below). Plants grown under condition of "continuous day."

it will be seen that the cycle may be as pronounced as in the previous cases, but that here, starting the test one-half cycle late shifts the time of maxima and minima by one-half of a cycle—i.e., it appears that when plants are grown under conditions of constant light, a twenty-four hour cycle is initiated by the process of decapitation.

The above curves represent data obtained in exudation tests made under constant high intensity light (cabinet A), but similar results were obtained

in constant low intensity white light (temp. baths) and ruby light (room C).

EFFECT OF TEMPERATURE.—Preliminary tests showed that exudation at 10°C. was so low that accurate measurements of rate were impractical. In an experiment presented as figure 5, "inverted day"

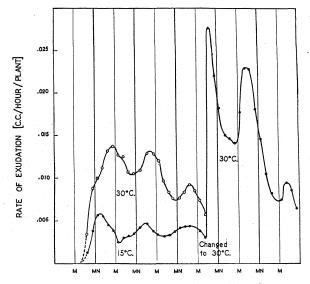


Fig. 5. Effects of temperature on rates of exudation.

plants were studied at 15° and 30°C. It is evident that temperature has a marked effect on the rate of exudation. At 30°C., exudation rate climbed to a high level and then fell rapidly, while at 15°C. the rate maintained a low value for some time. There are minor differences in the time of maxima and minima, but it seems possible that these may be due to secondary effects resulting from the difference in magnitude of the rate of exudation. The length of the cycle is only slightly, if at all, influenced by the temperatures at which the plants were tested. After three distinct maxima had been observed, the plants were transferred from 15° to 30°C. (the temperature of the solution culture being raised 15°C. in thirty minutes). This treatment resulted in an immediate maximum exudation, and subsequent maxima occurred at twenty-four hour inter-The general level of exudation was higher under these conditions than when the plants were placed directly into the 30°C. bath. The transition from 20° to 30° did not cause as large a stimulus as the sudden transition from 15° to 30°C. did. Plants grown in the greenhouse gave essentially similar results.

#### SUMMARY

Decapitated *Helianthus* plants show a definite autonomic cycle of exudation.

During the growth of these plants the twentyfour hour environmental cycle of a normal day or a twenty-four hour cycle consisting of successive twelve hour artificial light and dark periods can fix the time of day when the maxima and minima of the twenty-four hour exudation cycle occur. Under these conditions the exudation cycle is not materially affected by decapitation or slight temperature changes.

Plants grown under constant conditions show twenty-four hour autonomic cycles of exudation, but in this case the time of decapitation determines the time of occurrence of maxima and minima. A drastic rise in temperature during the exudation period will also determine the time of occurrence of maxima and minima.

The twenty-four hour periodicity of the exudation cycle is not materially affected by temperature and other treatments described.

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## SOME EXPERIMENTS ON BUD GROWTH 1

#### F. W. Went

The literature on bud inhibition has recently been summarized in an excellent review by Thimann (1939); so a discussion of the different viewpoints on lateral bud growth can be omitted. The following experiments all bear on this problem and allow a further critical consideration of some theories.

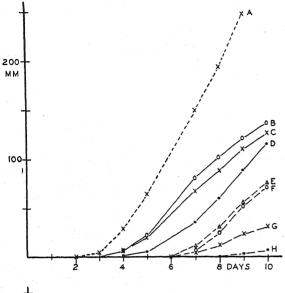
All experiments were carried out with etiolated stems of peas, *Pisum sativum* var. Alaska. They were grown in washed sand in a dark room at 24°C. and 85 per cent humidity and were used approximately seven days after planting, when the third internode was almost full grown. For the experiments the growth of the bud in the axil of the first or second bract was used. Care was taken to select only plants which were of the same length and development.

INCREASE OF INHIBITION WITH DISTANCE.—The results of the first experiments confirmed Snow's experiments on the increase of inhibition with increasing distance from the source of inhibition. Snow (1931a) in his experiments used the effect of the apical bud or leaves. But here this effect was replaced by auxin paste. Figure 2 clearly demonstrates that simple decapitation at various distances from the bud did not appreciably influence the outgrowth of the lateral buds. Neither did the distance have much effect when very low auxin concentrations were applied, which increase lateral growth compared with pure lanoline. This proves that the wound as such has little to do with it. But the higher auxin concentrations (0.2 and 1 mg. indole acetic acid per gram lanoline) were much more effective when the paste was applied 50 mm. instead of 5 mm. above the lateral bud (fig. 1, 2).

<sup>1</sup> Received for publication October 1, 1938.

A significant observation can be made by calculating the growth of the inhibited plants of figure 1 in a different way. If the growth curves of the individual buds inhibited with the different indole acetic acid concentrations are compared with those of the control buds treated with pure lanoline, the points will practically coincide by changing only the time axis (see fig. 3). This means that for several days (two to four, or more) the buds were completely inhibited and that the inhibition was then almost instantaneously and completely released. It even seems that the initial growth of the buds was slightly more rapid when they had first been inhibited. The supply of auxin from the lanoline paste at the cut surface was more or less continuous, and only gradually it decreased, so that this phenomenon of sudden release of inhibition cannot be explained by a sudden change in auxin concentration. Besides, this release of inhibition occurred sooner the closer the bud was to the auxin supply. In figures 1 and 2 the ultimate growth rate of the inhibited buds does not seem to parallel the growth rate of the controls, but this is due to the fact that not all inhibited buds started to grow simultaneously, and some were still dormant at the time of the last growth measurement.

The effect of distance on bud inhibition was also studied with other compounds, which were not so effective in growth or even had no growth activity at all, but which gave the preparatory reaction in the pea test (hemi-auxins). Thimann (1935) had already reported that substances able to cause growth but deficient in transportability (indene acetic acid and coumaryl acetic acid) caused strong bud inhibition when applied close (7-9 mm.) to the bud but had little effect when applied



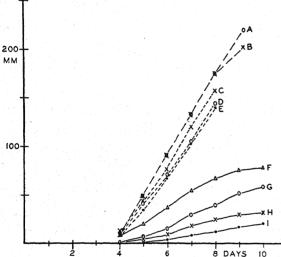


Fig. 1 (above). Relation between bud length (ordinate) and time (abscissa) after decapitation and application of pure lanoline (A), indole acetic acid 0.2 mg/g lanoline (B, C, D), and 1 mg/g (E, F, G, H). Decapitated 5 mm. (B, E), 15 mm. (B, F), 30 mm. (A, C, G), and 50 mm. above bud (D, H).

Fig. 2 (below). Relation between bud length (ordinate) and time (abscissa) after decapitation and application of pure lanoline (C, D, E), indole acetic acid 0.05 mg/g lanoline (A, B), and 0.2 mg/g (F, G, H, I). Decapitated 5 mm. (A, F), 15 mm. (A, D, G), 30 mm. (B, C, H), or 50 mm. (B, E, I) above bud.

50-60 mm. above the bud. And Van Overbeek (1938) found the same thing for  $\gamma$  phenyl butyric acid, a hemi-auxin. The experiments carried out by the writer on many thousands of peas were too variable to present all data in detail, but table 1 summarizes the results. Lower concentrations of all these substances had no effect, with hydrocinnamic acid no inhibition was found at 4 mg/g and lower, benzoic acid was inactive in 4 mg/g. In all

Table 1. Decrease of bud inhibition when different concentrations of substances are applied at various distances from the bud.

	Concentra-	Distand bu	
Substance	lanoline	10 mm.	30 mm.
Indole acetic acid	. 1 mg/g	+.+	+++
Indole propionic acid	$\begin{array}{cc} 4 \text{ mg/g} \\ 2 \text{ mg/g} \end{array}$	++	
Indole butyric acid and Anthracene acetic acid	$\begin{pmatrix} 2 \\ 0.4 \end{pmatrix}$	++ ++	
Phenyl acetic acid	$20  \mathrm{mg/g}$ $2  \mathrm{mg/g}$	+++	0
Phenyl butyric acid	5  mg/g 2  mg/g	+ ±	0 0
Cis-cinnamic acid	. 20 4	++	
Cyclo-hexane acetic acid	. 20 2	++	

+++> 80 per cent inhibition. ++ 30-70 per cent inhibition. + 10-20 per cent inhibition.

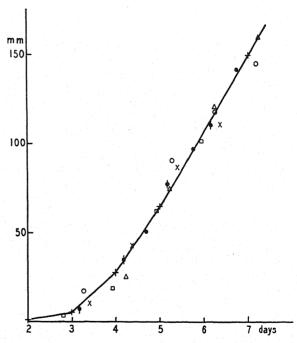


Fig. 3. Growth of lateral buds (ordinate: length; abscissa: days after decapitation) on decapitated shoots treated with pure lanoline (drawn line, same data as fig. 1, curve A). Over this line are fitted by shifting along the abscissa, the growth curves of lateral buds which were inhibited for 2–5 days by indole acetic acid pastes. Each dot, triangle, circle, etc., is the mean of several buds which started to grow at the same time. Triangle and cross come from plants of curve F, squares from curve E, dots from curve D, and circles from curve E on figure 1.

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cases in which inhibition was only partial, some buds grew at a normal rate, whereas others were strongly inhibited. As far as could be determined, this was not due to a lack of contact between paste and the apical cut surface.

In this connection some other experiments have to be mentioned. When cis cinnamic acid 10 mg/g or phenyl butyric acid 10 mg/g were applied 5 mm. above the third node, the bud on this node was strongly inhibited. But soon the bud on the second node started to grow out, and then the higher bud was inhibited from two sides. What happened to the auxin coming from the upper bud that it did not inhibit the lower one? This is partially answered in an experiment, reproduced in table 2, in

Table 2. Auxin content by ether extraction, in degrees Avena curvature (with standard deviation), of buds and 30 mm. of stem of pea plants 56 hours after decapitation.

Decapitated stems treated 56 hours with	Stem	Bud	Total
Lanoline Indole acetic acid	$9.5 \pm 0.7$	$9.8 \pm 0.8$	19.3 ± 1.1
0.05 mg/g Phenyl butyric acid	$10.8 \pm 1.0$	$10.7 \pm 0.9$	$21.5 \pm 1.3$
2 mg/g	$6.5\pm0.6$	$15.5\pm1.1$	$22.0 \pm 1.2$

which the bud and 30 mm. of adjoining stem were analyzed for auxin by extracting them with ether. It will be seen that lanoline and a low applied auxin concentration gave the same amount of auxin. But although the total amount of auxin present in the plants inhibited with phenyl butyric acid was the same, its distribution was different, since more was present in the bud and less in the stem. This is an indication that less auxin was transported downward, and thus the inhibition of the lower bud through the upper would be smaller. The same decrease in auxin content of the stems treated with

phenyl butyric acid was found by Van Overbeek (1938).

RELATION BETWEEN AUXIN AND BUD GROWTH FAC-TORS .- In order to find out something more about the relation between the inhibiting substances and the bud growth factors, another technique was used in the following experiments. Pea stems were decapitated at a certain distance above the bud whose growth was to be studied. Then lanoline containing various concentrations of indole acetic acid or y phenyl butyric acid was applied to the cut surface and left for periods ranging from 24-48 hours. Then the extreme apex with the lanoline paste was removed, the stems were cut 3 cm. below the bud and placed in vials containing 1 cc. of a 2 per cent sucrose solution. In the next few days the buds started to grow out, but very soon they had exhausted the supply of bud growth factors, and growth stopped. Measuring of the bud length after 5-7 days allowed the approximate determination of the amount of bud growth factors which were present in the piece of stem at the moment of placing the stem with bud in the sugar solution.

When pieces of stem were cut off immediately after decapitation, practically no bud growth occurred. On some days this residual growth was somewhat more than on others. But when the peas were left decapitated for one or two days, the buds not only started to grow out, but also showed considerable further growth when the stems were cut off and placed in sugar solution. (See table 3.) When the peas were cut immediately after decapitation, the buds did not become visible and measurable in this experiment. But from then on each 12 hours longer they were left on the plant practically doubled their ultimate length. This was not due to the difference in initial length, since between the 12 and the 48 hour group there was at most a 0.5 mm. difference in bud length at the moment the stems were cut off. It was due rather to a greater growth response.

Table 3. Length of buds in mm. on isolated pieces of etiolated pea stems, 2, 4, and 7 days after cutting them off 30 mm. below the bud and placing them in 2 per cent sucrose. The peas were decapitated 10 mm. above the bud 12, 24, 36, 48, or 60 hours before cutting them off. Immediately after decapitation, paste was applied. Each figure represents the mean length of 15-80 buds.

WENT-BUD GROWTH

	David often	Time interve	al between o	decapitatio	and cuttin	g off stems
Treated with	Days after cutting	12 hrs.	24 hrs. S	36 hrs.	48 hrs.	60 hrs.
Pure lanoline	2	0.2	0.6	1.6	$2.8 \pm 0.17$	$4.6 \pm 0.69$
	4	$0.6 \pm 0.10$	$0.9 \pm 0.22$	2.5	4.2	7.4
	7	$1.7 \pm 0.14$	1.3	2.7	4.7	8.0
Indole acetic acid.						
0.05 mg/g lanoline	2	0.2	0.8	1.3	3.2	$5.8 \pm 0.68$
	4	0.5	1.2	2.1	4.5	8.2
	7	0.5	1.5	2.7	4.9	8.6
Phenyl butyric acid,						
2 mg/g lanoline	2	0.6	0.9	1.4	2.0	4.0
	4	$1.0 \pm 0.14$	$1.5 \pm 0.21$	2.5	3.2	6.5
	7	$1.1 \pm 0.12$	1.7	2.5	3.6	7.1

Table 4. Length of lateral buds in mm. above bract, when pea stems were decapitated and treated with different lanoline pastes, 48 hours later cut off 30 mm. below the bud and placed with their bases in 2 per cent sucrose solution.

Decapitation				20 mm	. abo	ve bu	d		
After 48 hours	Ste	em left	above l	oud	-	St	tem cut		oud
Measured after days	0	2	4	6		0	2	4	6
Lanoline control	0.8	3.0	3.8	4.0		1.0	1.7	2.5	2.9
Indole acetic acid 0.05 mg/g	1.0	5.8	7.4	8.2		1.0	2.5	3.1	4.0
Indole acetic acid 0.2 mg/g		2.9	3.1	3.7		0.9	1.6	2.1	2.7
Indole acetic acid 1 mg/g	0	0	0.3	0.3		0.1	0.4	0.6	0.7
Phenyl butyric acid 2 mg/g	1.0	5.0	7.6	8.7		1.0	2.0	2.5	2.7

The ultimate cessation of growth was not due to a lack of carbohydrates nor to an auxin shortage, since application of a very low concentration of indole acetic acid (0.02 mg/g lanoline), which in intact plants would not appreciably affect growth, slightly inhibited the bud growth when applied to the apical cut surface of isolated stems (in 3 groups 5.4, 5.0 and 4.2 mm. against 7.6, 7.6 and 7.3 mm.). Neither did application of 100 or 10 mg. tryptamine per liter 2 per cent sucrose solution increase bud growth.

Table 4 gives the data of an experiment which was planned to determine what auxin application actually does to the bud growth factors. Peas were decapitated 20 mm. above the bud, and paste was applied. Forty-eight hours later the paste was removed and the stems all cut 30 mm. below the bud. The stems of one-half the group were cut off immediately above the bud. Then they were

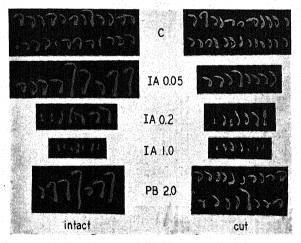


Fig. 4. Length of lateral buds one day after the last measurement of table 4. Pea stems decapitated 20 mm. above lower lateral bud and lanoline paste applied; C = pure lanoline, IA = indole acetic acid, 0.05, 0.2, and 1.0 mg/g lanoline, PB = phenyl butyric acid 2 mg/g. Two days later all stems were cut 30 mm. below lower bud. Those marked "intact" had only the paste removed from the stem apex (column A, table 4), from those marked "cut" the stem above the bud was cut off (column B, table 4).

placed in small vials in a 2 per cent sucrose solution. At that time no difference in the development of the buds could be noticed, except with the highest indole acetic acid concentration, which so completely inhibited the buds that they were not visible between the bracts.

There is a remarkable difference between groups A and B (see also fig. 4). When the 20 mm. piece of stem which was left after decapitation was cut off before the buds started to grow, the buds grew considerably less than when it was left on, which means that this piece contained a considerable amount of bud growth factors. And it contained much more of these when the tops had been treated with y phenyl butyric acid or a low indole acetic acid concentration than the lanoline controls. Further, an auxin concentration which caused a considerable bud inhibition (0.2 mg/g; see fig. 1, 2) did not have such an effect when the paste was removed after two days. Figure 4, which gives a clear picture of this experiment, shows the shadow picture of the buds six days after cutting the stems off.

In another experiment of the same type the buds were weighed. The stem was decapitated 10 mm. above the bud, and two days later cut 30 mm. below and immediately above the bud. Eight days later the weight per bud was:

Lanoline control 2.9 mg	2.9 mg
Indole acetic acid 0.05 mg/g	3.6 mg
Indole acetic acid 0.2 mg/g	
Indole acetic acid 1 mg/g	
Phenyl butyric acid 2 mg/g	5.3 mg
Phenyl butyric acid 5 mg/g	$4.0  \mathrm{mg}$

In this experiment the buds grew out to 0.7 mg. only when the stems were cut immediately after decapitation.

Because of the importance of these experiments in discussing the problem of bud inhibition, still another experiment is presented in table 5.

The results closely parallel those of table 4. In another experiment the effect of phenyl butyric acid 2 mg/g of increasing subsequent bud growth was found only when the stems were decapitated 7 mm. above the bud, but not with 20 mm., as was found in the experiment of table 4.

Table 5. For explanation see table 4.

Decapitation	5	mm. al	bove bu	d	20 mm. above bud			
After 48 hours	Cut above		Not	cut		just e bud	Not	cut
Measured after days	2	6	2	6	2	6	2	6
Lanoline control	1.6	1.7	1.1	1.4	1.2	1.4	1.9	2.3
Indole acetic acid 0.02 mg/g	1.5	1.6	1.4	1.8	1.4	1.4	2.6	3.4
Indole acetic acid 0.05 mg/g	1.2	1.3	1.1	1.3	1.4	1.5	3.0	3.7
Indole acetic acid 0.10 mg/g		1.1	0.7	1.0	1.7	1.9	2.2	2.6
Indole acetic acid 1.0 mg/g		0.4	0.2	0.2	0.2	0.2	0	0

In the experiment of table 3 also data on the optimum indole acetic acid concentration were obtained. When treated with pure lanoline the bud length was  $2.8\,\pm\,0.17$  mm.

With indole acetic acid 0.014 mg/g

bud length was 3.0 mm.

With indole acetic acid 0.02 mg/g

bud length was 3.4

With indole acetic acid 0.033 mg/g bud length was 3.5  $\pm$  0.21 mm.

With indole acetic acid 0.05 mg/g

bud length was 3.2 mm.

With indole acetic acid 0.1 mg/g

bud length was 1.8 mm.

From these figures an optimum concentration of 0.03 mg. per gram lanoline can be calculated; table 4 indicates 0.05 mg/g. The difference between these figures is not very large, and the most important point is that in each case there was a considerable increase in bud length when the peas were pre-treated with low auxin concentration, provided that 5-20 mm. of stem was left above the bud. The fact that when this part of the stem above the bud was cut off no appreciable increase in bud growth occurred indicates that a difference in length of the bud at the moment of cutting of the stem was not responsible for the increased growth later, of which effect the 0 hour measurements of table 4 give a direct proof. Furthermore,

when the growth rate for equal bud lengths was calculated, it was always greater for the auxin treated stems than for the controls.

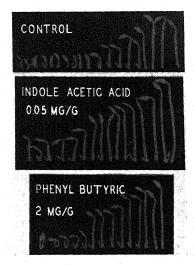


Fig. 5. Lateral buds developed on pea stems, cut off 30 mm. below the bud two days after decapitation 10 mm. above this bud. Lanoline pastes were applied to the apical cut surface during these two days. Measurements of these buds two days earlier are recorded in the last column of table 6.

Table 6. Each figure is the mean length in mm. or weight in mg. of 8-10 lateral buds 5 days after decapitation. The peas were decapitated 10 mm. above the second (lower bud) or third (upper bud) internode, and lanoline paste was applied to the cut surface. One or two days later the paste was removed by cutting off the extreme 2 mm. of the stem, the stems were cut 30 mm. below the bud (lower or upper) and placed in 2 per cent sucrose solution.

	Cut off 1	lay after decapitation	Cut off 2 da	ays after decapitation
	Lower bud	Upper bud	Lower bud	Upper bud
	Length	Length Weigh	t Length	Length Weight
Lanoline control Indole acetic acid	1.1	2.45	$2.1 \atop 2.4 $ $2.3 \pm 0.3$	8.7 $8.8$ $19.2$ $10.2$ $10.2$ $10.3$ $10.4$
0.05 mg/g lanoline γ Phenyl butyric ac 2 mg/g lanoline		$3.7$ $3.6 \pm 0.4$ 5.3 $3.7$ $3.6 \pm 0.4$ 4.4	$4.0 \pm 0.8$ $1.9$	$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$

Table 7. Etiolated pea stems were decapitated 8 days after germination 20 mm. above the second bract, and lanoline paste was applied to the cut surface. Two days later half the plants of each group were cut off 30 mm. below the second bract, and the auxin paste was removed. The other half was left as they were, as a control for normal bud growth. Bud length 3, 6, 8, and 10 days after decapitation.

<u> </u>			Measurer	nent after		0 11 6
1n	dole acetic acid in mg/g lanoline	3 days	6 days	8 days	10 days	Growth from 3-6 days
Stems left on plant	. 0	3.0 mm.	87 mm.	150 mm.		84 mm.
	0.02	3.2	73	131		70
	0.05	1.3	57	109		56
Stems cut off 30 r	nm. 0	3.6	4.7		5.0 mm.	1.1
below bud	0.02	3.3	8.8		10.1	5.5
	0.05	2.3	6.1		6.2	3.8

The magnitude of this effect of auxin on the distribution of bud growth factors varies from experiment to experiment. In only one experiment was the effect so small that it might be questioned. In all other experiments (seven, each comprising 200–450 plants) an effect was clearly noticed. In the sixth experiment the increase in bud growth by a previous treatment with 0.05 mg/indole acetic acid per gram lanoline was statistically highly significant (see table 6 and fig. 5), the difference between treated and control plants being more than three times the standard deviation.

In another experiment the determinations described above were combined with direct measurements of bud inhibition when the stems were not cut off at the base. As table 7 shows, an indole acetic acid concentration which caused bud inhibition as long as the stems were attached to the plant had very definitely increased the amount of available bud growth factors in those stems. Thus the simultaneous occurrence of bud inhibition and accumulation of bud growth factors was proven.

Lower shoots inhibiting more apical shoots. -The following observations are of interest in connection with the fact that in decapitated peas the most apical bud grows out when the cotyledons are present, but when the cotyledons are cut, the basal or even cotyledonary buds will develop (Went, 1938a). When a pea stem is cut off, it will not grow any more, nor will lateral buds develop, until a functional connection with a new root system has This can be done either by been established. grafting the stems on another root system or by letting adventitious roots develop. In the latter case the amount of storage food seems to be inadequate to cause much growth, and only when a leaf is present and the plants are exposed to light does much bud growth take place. But when the shoots are grafted, and a graft union is established, the apical and lateral buds can renew growth (Went, 1938b). If before the formation of the graft union lateral buds below the graft start to grow out, they completely inhibit growth of the grafted shoot. But one or two days after cutting off these laterals the grafted shoot will grow at a normal rate and then

inhibit the lower lateral buds. Thus we have a perfect case of correlative inhibition which is carried from base to top, such as Snow (1931b) has demonstrated to exist; and in the same stems an inhibition could be carried from top to base.

A more remarkable fact was observed in these grafting experiments. If the grafted shoot was rather long (50 mm. or over), and if a lateral bud was present near its basal cut surface, in some cases this bud did grow out after the graft had "taken," and in that case the apical bud was completely inhibited. From the measurements it appeared that sometimes the apical bud started to grow (very slowly) simultaneously with the lateral bud and very soon was completely inhibited by the growing lateral bud, but in other cases the lateral bud inhibited the apical from the beginning. This inhibition was not due to injury of the apical bud or inability to grow, for as soon as the lateral was removed, the apical bud resumed growth. In none of these experiments were indications of injury due to the inhibition by more basal growing buds observed, such as Snow describes (1931b). This may be due to the difference in method (Snow grew his plants in daylight) or the shorter time in the experiments reported above.

BUD GROWTH AND BUD INHIBITION ON ROOTS.—It was thought that more information about bud growth might be gathered from experiments with roots which regenerate buds on their basal cut surface. Many such roots are known (Cichorium, Cakile, Taraxacum), but the following preliminary experiments were performed with the fleshy roots of Oenothera macrosiphon. These plants were suggested for the experiments by Dr. S. H. Emerson, who had observed the abundant formation of adventitious shoots whenever a basal cut surface of these roots was exposed. The roots of a few plants were carefully washed, cut into pieces 8 cm. long, and combined into comparable groups of five pieces each. In general the very thick and the thinner (more apical) parts of the roots gave similar response.

The roots were left at 24°C. and 85 per cent humidity in a dark room, each one individually apex

Table 8. Number, total length, and total weight of the shoots, developed in 14 days on the basal cut surface of five pieces of Oenothera macrosiphon roots 8 cm. long. They were first treated for 5 days with indole acetic acid. Root formation: 1 very poor, 2 poor, 3 good, 4 very heavy.

Concentration of in dole acetic acid			surface cut fter treatme		1	Basal surfa	ce left inta	et
		Shoots				Shoots		
	Number	Length	Weight	Roots	Number	Length	Weight	Roots
Applied from base								
$200\mathrm{mg/l}$					33	43 mm.	18 mg.	3
100	73	196 mm.	144 mg.	2	0	0	0	3
50	45	280 mm.	268 mg.	.1	18	38 mm.	22 mg.	2
20					19	225 mm.	460 mg.	3
10					21	310 mm.	667 mg.	1
0	72	640 mm.	818 mg.	2	66	425 mm.	483 mg.	1
Applied from apex								
200 mg/l					89	1,000 mm.	1,600 mg.	4
100	103	920 mm.	1,116 mg.	4		1,375 mm.	2,133 mg.	4
0						1,120 mm.	2,118 mg.	3

down in a small bottle 6 cm. high, with a small amount of water. The indole acetic acid was supplied by immersing the extreme tip of apical or basal cut surface in the solution for from one to five days. Afterwards the base was cut or left intact. Two weeks later the shoot and root development was noted (see table 8). The following conclusions may be drawn:

The number of shoots developing is less when the basal side was immersed in the solution. Apparently this had a toxic effect. Renewing the cut surface did away with this inhibition.

When treated with indole acetic acid, the number of adventitious shoots developing was not greatly affected; their total length and weight, however, dropped off very much when treated with the higher indole acetic acid concentrations. This means that the treatment primarily affected the bud growth factors and not the initiation of buds.

Apical treatment of the roots with indole acetic acid increased only their root formation, but had little effect on bud development.

There seemed to be a more or less inverse relationship between root and shoot development on these root cuttings.

In another experiment basal treatment with high indole acetic acid concentration even caused roots to grow out from the base on two out of eight cuttings, whereas the bud formation was completely inhibited.

Discussion.—The facts presented in this paper form a good basis for discussion of some of the bud inhibition theories put forward by different investigators. Any theory trying to explain bud inhibition by a direct effect of auxin (Thimann, 1937) is at a loss to explain the increase of inhibition with distance. For on the one hand the inhibition is the more complete the higher the applied auxin concentration; on the other hand the inhibition becomes

stronger the farther away the buds are located from the place of application. For Snow's theory of a specific inhibitor formed under the influence of auxin this effect of distance gives no difficulties except that it has to be assumed that everywhere in the stem the inhibitor is formed with the transporting auxin. However, some substances which definitely do not cause growth (\gamma\ phenyl butyric acid) can inhibit buds, and therefore Snow's hypothesis (1937) would have to be modified. The diversion hypothesis (Went, 1936), which assumes that an apical supply of auxin causes the other bud growth factors (like caulocaline (Went, 1938a)) to move towards this supply, does not depend on the growth activity of substances for its validity. And the increase of inhibition with distance follows directly from the assumption made. For the effect of applied auxin would be to cause more and more caulocaline to accumulate in the upper part of the decapitated stem. This might be compared with the filling of a tube. As soon as the tube is full as far as the bud, it will overflow into the bud, and from then on the bud starts to grow at a normal rate. This overflow principle explains in the first place why there is an increase in inhibition with distance, since the longer the piece of stem to be filled up, the longer it will take, and the more complete the inhibition is. And in the second place it gives a good reason for the sudden release of inhibition. The latter effect can be explained equally well with Van Overbeek's (1938) blocking hypothesis.

Another conclusion can be drawn from the experiments. A high auxin concentration inhibits a bud for a longer period than a low concentration. Since the growth after release of the inhibition is the same, the flow of bud growth factors to the lateral buds is the same with both a high and a low auxin concentration. Therefore, the higher the applied auxin concentration, the higher the con-

centration of caulocaline can become in the portion of the stem between the bud and the auxin paste. Further experiments may disclose whether all or only part of the accumulated caulocaline can be recovered. In the growing apical bud the caulocaline is used in growth as soon as it arrives, and there are no indications that it accumulates. This may be the case only in dormant buds. Not enough facts are available to go deeper into this problem.

A very nice proof of the contention that under the influence of auxin, caulocaline, or bud growth factors in general, accumulate in the stem apical from the bud, is given in the experiments of tables 4, 5, 6 and 7. In these experiments a differentiation between two activities of auxin is made. In the first place a continuous supply of low concentration during the outgrowth of the bud decreases its growth. But on the other hand a pre-treatment with indole acetic acid greatly increases growth. If too high a concentration is applied, apparently enough remains in the piece of stem to inhibit further growth, but concentrations, causing inhibition when applied continuously, increase bud growth when the stem is treated for the first one or two days after decapitation. This fact is completely unexplainable on the basis of Snow's theory of the formation of a special inhibitor under the influence of the auxin action. The experiments seem simple enough, and are so completely reproducible as to be conclusive. Especially the experiment of table 7 is convincing, since the accumulation of bud growth factors is shown to occur under the influence of indole acetic acid, while in comparable plants the same concentration causes bud inhibition.

It seems that for these experiments the diversion hypothesis, among the existing theories, gives the only possible explanation. This hypothesis also furnishes a good explanation of the inhibition of higher lateral buds or even the apical bud by a rapidly growing lower lateral bud. This lower bud overtakes a more apical bud only when (a) the auxin supply from above is reduced, as in the grafting experiments, or (b) in the experiments in which the cotyledons are removed, or (c) when a substance which has only a limited transportability is applied. But once it has the lead, the apical bud does not have a chance as long as the basal bud is growing. The latter facts are difficult to explain on the basis of Van Overbeek's blocking hypothesis. For the vascular connections of the apical bud with the base of the plant are normally developed and, according to the hypothesis, could not be blocked for the transport of growth factors by auxin. Besides, very little auxin is present in these apical buds which are not growing.

A further discussion of bud inhibition theories does not seem necessary, with the review of Thimann (1939) at hand. A few words, however, might be added concerning the modification of Went's diversion hypothesis by Ferman (1938), who claims that not specific bud growth factors like

caulocaline, but the auxin precursor are diverted by the auxin, natural or applied. The data presented in table 2 and the fact that tryptamine, which can act as an auxin precursor (Skoog, 1937), has no effect on bud growth in excised pea stems are not in favor of Ferman's supposition. Also in other experiments with grafted peas no correlation between growth rate and auxin production was found (Went, 1938b) so that there neither the auxin nor the auxin precursor were the limiting factors for bud growth. Although the present author believes that not only calines but also the auxin precursor may be diverted under the influence of auxin (see e.g. Went, 1935), he does not believe that diversion of the auxin precursor underlies the phenomenon of bud inhibition. This is supported by the determinations of Van Overbeek (1938).

The experiments with bud growth on the Oenothera roots are interesting inasmuch as they show that the phenomenon of bud inhibition by auxin is the same in stems and roots. It does not primarily affect the initiation of shoots, but their subsequent growth. Another interesting fact is that the polarity in the auxin effect is very pronounced, which is noteworthy since hardly any polarity of auxin transport in roots was observed (Heidt, 1931; Gorter, 1932; Faber, 1936). It is quite clear that in this respect the polarity of the root is continuous with that of the shoot, as Went (1932) had postulated.

#### SUMMARY

In experiments with etiolated pea shoots the phenomenon of increasing inhibition with increasing distance between inhibited lateral bud and inhibiting agent (Snow, 1931a) is confirmed with application of indole acetic acid at different distances from the lateral bud. The inhibiting effect of some non-auxins and substances with low growth activity is described.

The most important experiments were carried out with short pieces of stem (30–50 mm. long), carrying one lateral bud. They were cut off the plant at various times after decapitation and application of various substances. The experiments show that the indole acetic acid and  $\gamma$  phenyl butyric acid caused an accumulation of bud growth factors just below their point of application and that they did not inhibit growth by themselves. The experiments show very clearly that the inhibition is a secondary effect of the applied auxin.

Some cases of inhibition of the apical bud by lateral buds are described.

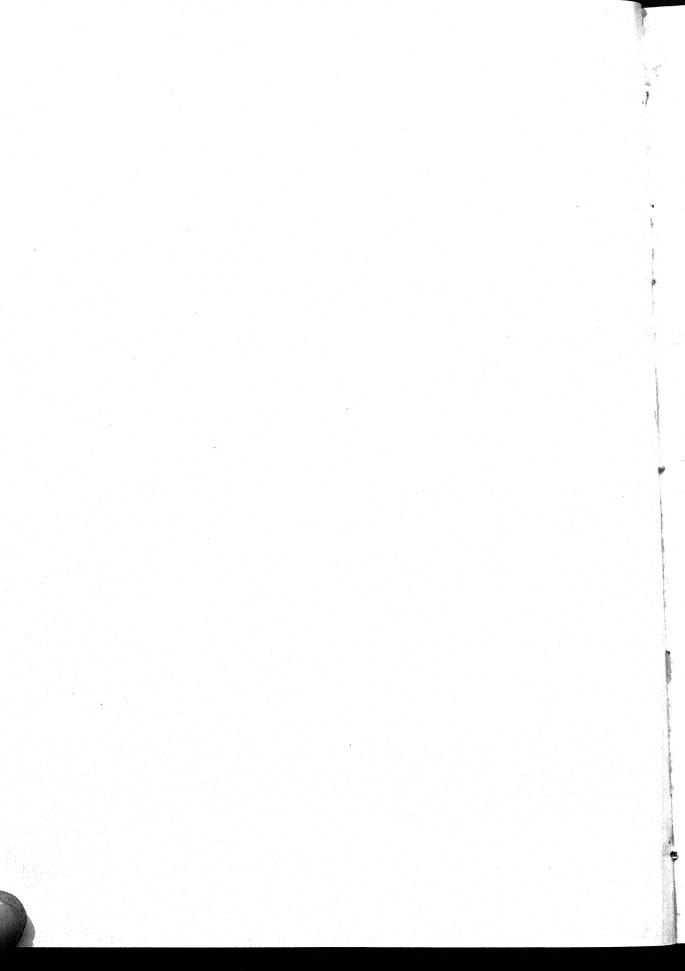
Finally, it is shown that bud inhibition can be brought about in roots which regenerate buds on their basal cut surface in much the same way that it can be brought about in stems.

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## A NEW CONIDIOBOLUS WITH SEXUAL REPRODUCTION 1

John N. Couch

THE GENUS Conidiobolus was founded by Brefeld (1884) on C. utriculosus and C. minor, which were overgrowing the fruit bodies of certain jelly fungi. Both species were accidentally obtained while Brefeld was trying to get cultures of Hirneola and Exidia from spores by placing the fruit bodies over slides on which were drops of water and nutrient solutions. Such fruit bodies were placed under bell jars, and on the second day Brefeld discovered the large conidia-bearing mycelium growing on his slides and overrunning the germinating spores of the jelly fungi. At first Brefeld looked upon the fungus as an interloper and a pest, but later, upon seeing the peculiar Entomophthora-like conidia, he became interested, isolated the fungus, and kept it in culture in dung decoction for over six months, during which time his studies were made. Brefeld described in great detail the vegetative structure, conidial formation, and sexual reproduction in C. utriculosus. The sexual reproduction is of particular interest. Gametangia of unequal size conjugate, the zygote being formed in the swollen part of the larger cell.

Up to the present time, we know nothing of the sexuality of *Conidiobolus* except from Brefeld's original description of *C. utriculosus*. The chief object of this paper is to describe a species of *Conidiobolus* in which the same type of sexual reproduction occurs and to discuss the validity of the genus in the light of more recent researches.

The fungus described below appeared as a contaminant, May 13, 1935, in a Petri dish on maltosepeptone agar with spores shot from Septobasidium apiculatum on Cornus amomum. It is impossible to be certain what the fungus was growing on in nature, perhaps the Septobasidium or a small insect or perhaps (and this is very unlikely) the Conidiobolus was brought in by air currents from an unknown source.

Conidiobolus Brefeldianus sp. nov.—Mycelium thread-like or breaking into hyphal bodies, depending upon the culture media. On maltose-peptone agar No.  $5^2$  forming an intramatrical disc, the outer zone of hyphal ends being full of vacuolate protoplasm and alive, while the older hyphae are mostly dead and empty of protoplasm. Hyphae wavy or straight,  $5.4-8.4\,\mu$  thick, branched, septate, the cross wall usually thick and conspicuous, as a rule without constrictions at the septa; the segments, variable in length from about 50 to several hundred microns long, usually  $300-420\,\mu$ . Protoplasm with numerous conspicuous granules and rounded or elongated vacuoles. Conidiophores unbranched, positively phototrophic, stalk usually distinctly swollen in the middle, usually  $7-10\,\times\,30-50\,\mu$ , but sometimes much longer; each conidiophore giving rise

<sup>1</sup> Received for publication November 30, 1938. Presented before the Mycological Society of America,

Atlantic City, December, 1936.

to one conidium. Conidia spherical, 10-31  $\mu$  thick, mostly about 20  $\mu$  thick, with a conical apiculus about 7.5  $\mu$  wide at the base and 5-7.5  $\mu$  long on conidia of average size. Conidia forcibly discharged by the sudden out-pushing of the papilla. Columella cone-shaped, persistent. Sexual reproduction by the conjugation of spherical gametangia of unequal size which usually arise from the adjacent ends of two segments in the same thread, or sometimes from two parallel threads or from two branches of the same thread. Smaller gametangia 9-12.6  $\mu$  thick when mature. Zygote formed in larger gametangium, spherical, 18-33  $\mu$  thick, usually about 23  $\mu$  thick, with smooth, thick, three-layered (?) wall, and one large, conspicuous, eccentric oil (?) globule which is outside the protoplasm; capable of germinating after a rest period of two weeks or more.

Conidiobolus Brefeldianus.—Mycelio ramoso, septato, rare fragmentario; hyphis 4.2–10  $\mu$  latis, segmentis 50 usque ad aliquot centena  $\mu$  longis; conidiophoris erectis, positive phototropicis, 7–10  $\times$  30–50  $\mu$ , simplicibus, clavatis, solitario conidio in apice; conidiis globosis, hyalinis, levibus, 10–25  $\mu$  latis, distincta conica papilla, valide ejectis; sporis perdurantibus (zygosporis) e copulatione laterali vel scalariformi cellularum hypharum efformatis; zygosporis intra cellulam in qua plasmata se jungunt efformatis, globosis, 21–33  $\mu$  latis, excentrico guttulo et episporio levi hyalino maturitate.

This species may readily be distinguished from C. utriculosus Brefeld by structural differences and by its much smaller size throughout. In C. utriculosus the conidia are pear-shaped (Brefeld's figures are more nearly ovoid with a short papilla), smooth,  $35 \mu$  wide by  $50 \mu$  long; while the conidia in the present species are spherical, smooth, 10-31 µ thick, most about 20 µ thick. Also in C. utriculosus the zygotes are 60-100  $\mu$  thick, most about 80  $\mu$  thick, and are furnished with a minutely warted almost colorless or bright yellow wall, while in the present species the zygotes are  $20-33 \mu$  thick, most about  $23 \mu$  thick, and have smooth walls. Brefeld gives no measurements, but the mycelial threads in his species appear from his drawings to be larger than in the present one. Brefeld cultured his fungus only in dung decoction, and it is possible that the numerous, short, lateral out-pocketings as well as the absence of septations in the young mycelium were due to the culture conditions. Brefeld's second species, C. minor, is based on a few small conidia observed in cultures of C. utriculosus. Martin (1925) suggests that these conidia were doubtless small ones of C. utriculosus reduced in size by repeated germinations in old cultures.

EXPERIMENTS WITH VARIOUS CULTURE MEDIA.—Considerable experimenting has been done to determine the different types of food substance utilized by Conidiobolus Brefeldianus and the character of growth of the fungus on different substrata. Only the more significant results are here summarized.

 $<sup>^2</sup>$  1,000 cc. H2O, 20 grams agar, 3 grams maltose, 1 gram meat peptone.

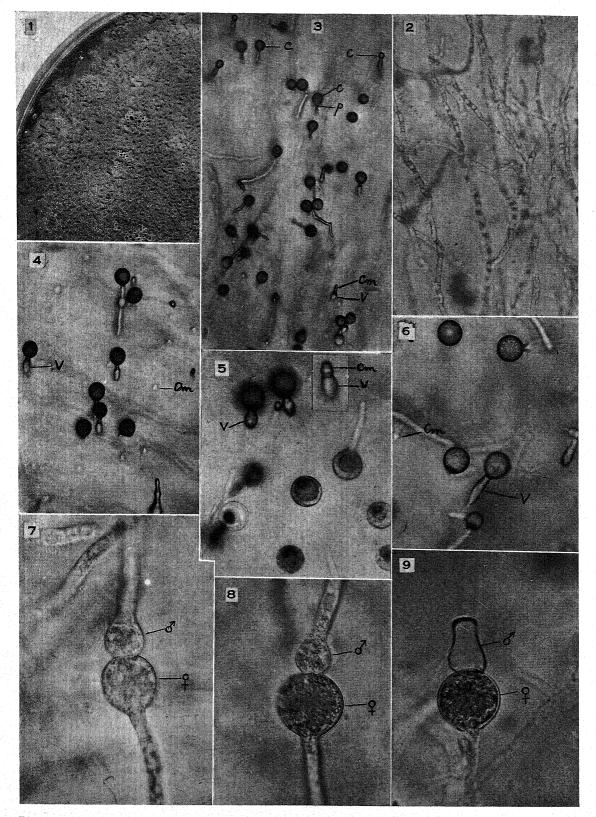


Fig. 1-9.—Fig. 1. Showing convoluted growth on sterilized egg yolk. Slightly enlarged.—Fig. 2. Mycelium on agar No. 35. Note vacuolate cytoplasm. A few septa are visible. Dark spots are conidia out of focus. Threads are all within agar. ×150.—Fig. 3. Conidiophores of different ages grown in dark chamber with small side opening.

The fungus will grow on a wide range of vegetable and animal matter. On cooked vegetables, such as carrots or sweet potatoes, growth is luxuriant, while on raw vegetables it is very poor. No growth occurred on cooked or raw fruits or fruit juices such as Winesap apples, Concord grapes, and Crataegus fruits. Growth on sterilized egg yolk (fig. 1) resembles the type of growth shown by Sawyer (1929, fig. 5) for Empusa sp. and thus tends to emphasize the physiological relationship between Conidiobolus Brefeldianus and Empusa sp. On the other hand, C. Brefeldianus failed to grow on any of the sterilized meats such as veal, pork, or lean beef, while Sawyer (1929) was able to culture Empusa sp. on such substrata. In horse dung decoctions of varying strengths our fungus produced fair growth. The threads were straight without the outpocketings described by Brefeld as very characteristic of C. utriculosus in the same media. Furthermore, in this culture medium our fungus produced very few conidia and no zygotes, while in the same culture medium such structures were abundantly formed in C. utriculosus. The conidial types on raw egg albumen are worthy of mention. On this medium many secondary conidiophores and conidia were formed. In some instances the primary conidium gave rise to a number of short stalks, on each of which was borne a small conidium (fig. 25, 26, 29), thus resembling the secondary conidiophores of Delacroixia coronata as described by Costantin (1897), Gallaud (1905), and Kevorkian (1937). Frequently the primary conidium gave rise to a long secondary conidiophore bearing on the swollen end 3 to 7 small conidia, each on a short, slender stalk, the structure strikingly resembling a basidium (fig.

In experiments with living insects one individual of an unidentified species of small hymenopterous wasp was infected, and also several gnats. Another species of hymenopterous wasp was uninfected after being in contact with the fungus for several days. Fruit flies (Drosophila melanogaster) on slices of bananas in contact with the fungus were uninfected and perfectly healthy after two weeks. The fungus also failed to infect termites, the latter making tunnels in the agar and living for several days until the cultures were overgrown by Penicillium.

Conidial formation.—Given a healthy mycelium on a favorable culture medium, the formation of conidia seems to be determined by proper aeration, a condition corroborated by Berdan (1937, 1938) in this laboratory for several species of Ancylistes.

Conidia are formed only if part of the mycelium is at the surface. On agar the threads at or close to the surface give rise to most of the conidiophores, but in such a culture conidiophores may also arise from threads deep within the agar. On the other hand, if the culture is entirely submerged in liquid media, no conidiophores are formed. However, if such a culture is supported at the surface, conidiophores are formed in about an hour's time.

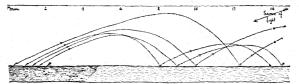
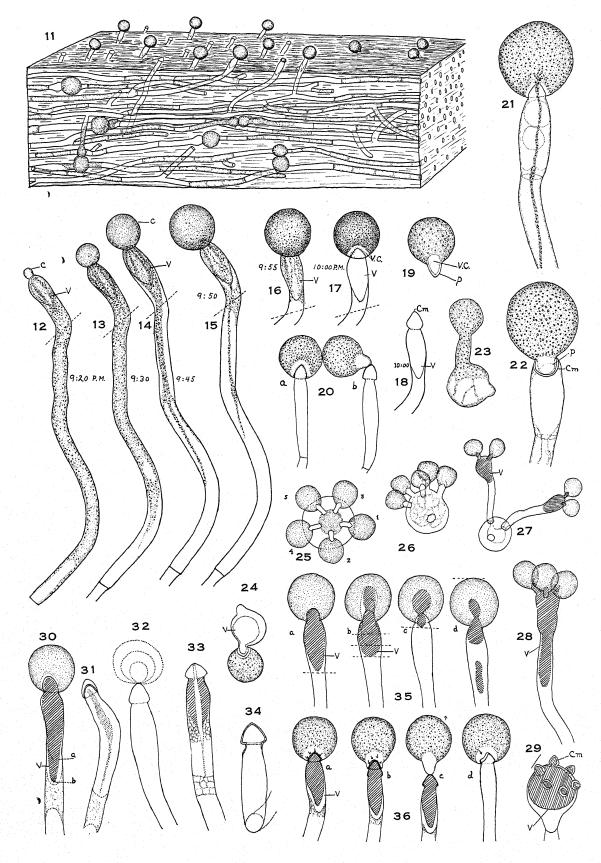


Fig. 10. Diagrammatic representation of the discharge of conidia on an agar plate, darkened save for one small source of lateral illumination. The direction from which the light comes is indicated by an arrow. The conidia, when discharged, may form secondary conidiophores, the secondary conidia being discharged again, and thus the conidia march across the plate toward the light. As indicated by the scale of millimeters, the conidia travel from 5 to 12 millimeters with each discharge. × about 100.

At first it seemed evident that the formation of the conidiophores was determined by light.3 In the first place, when cultures are kept out in the room and thus subjected to alternating daylight and darkness, they show a distinct concentric zonation, a new zone being formed each twenty-four hours; and in the second place, the conidiophores are positively phototropic. It has been found, however, that the formation of conidia is determined primarily by the nutrient material and only secondarily by light, thus corroborating the work of Lendner (1897). However, the number of conidia finally formed in alternating daylight and darkness is several times greater than in either continuous darkness or continuous light. Although conidia are formed on a great variety of culture media, certain media are far more favorable for their formation than others, as, for example, potato dextrose agar and a modification of Blakeslee's "230" in which the sugars are reduced by half (2 per cent agar, 1 per cent glucose, 1 per cent dextrose, 0.1 per cent peptone), whereas on agar No. 35 (see below for formula) practically no conidia are formed in total darkness and only a few in alternating darkness and

<sup>3</sup> The writer is indebted to Mr. George Christenberry for help with some of the experiments on the effect of light.

Conidiophores all directed toward source of light. ×150.—Fig. 4. Another region from same culture showing positive phototrophism of conidiophores. Note whitish columellae of old conidiophores and collapsed stalks. ×200.—Fig. 5. Note two conidiophores at top left each with a conspicuous vacuole in stalk. ×512. Small insert to right of two conidiophores shows columella and vacuole just after conidium had been shot. ×700. Also note germinating conidium and dark areas (vacuoles) in conidia toward middle and lower part of figure.—Fig. 6. Conidia, the large one at top right with collapsed stalk, the one just below middle with rigid stalk. Note collapsed conidiophore with conspicuous whitish columella on extreme left of figure. Young conidiophore on extreme right center before conidium has appeared. ×400.—Fig. 7-9. Stages in formation of zygote, smaller gametangium above larger below. In figure 9 small gametangium has completely emptied into larger. ×700.



light. In general it appears that media rich in carbohydrates favor the formation of conidia, while media rich (but not too rich) in peptone favor the formation of zygotes.

Under optimum conditions the formation of conidia may proceed very rapidly, the entire process from the upward growth of the conidiophore to the final ejection of the conidium occupying from 50 minutes to two hours. Any segment close to the surface or deep in the agar except one destined for sexual reproduction may produce a conidiophore. The first indication that a segment is to produce a conidium is the growth of one of the ends, or of a lateral branch up to and slightly beyond the surface. The projecting part may be considered the conidiophore and not infrequently may be separated from the basal part by a cross wall. The protoplasm flows into the conidiophore, which enlarges into a club-shaped structure. The conidium itself arises as a bud from the tip of the conidiophore (fig. 3, 12).

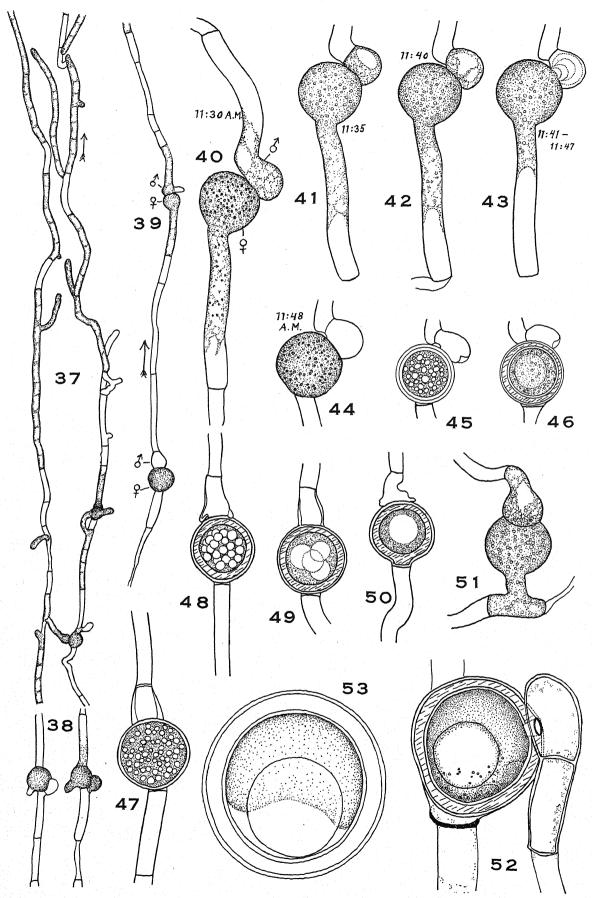
At an early stage in development a large, elongate, ovoid vacuole appears in the distal part of the conidiophore (fig. 5, 12). This structure is stained a yellowish orange with neutral red (1 part in 1,000  $\rm H_2O$ ) and extends up into the columella, persisting until the conidium is discharged and the conidiophore collapses (fig. 5, 6, 18, 30).

The protoplasm seems to flow up around the vacuole to pass into the conidium (fig. 12-16).

The movement of the protoplasm is fast enough so that one can easily observe the flow. As the conidium increases in size, the vacuole also enlarges. In figure 12 the conidium appeared as a minute bud at 9:20 p.m., and forty minutes later all the protoplasm had moved up into it (fig. 17).

After the conidiophore has become practically empty of its protoplasm, the vacuole stands out with striking clarity (fig. 5, 17, etc.). The nature of this vacuole is very puzzling. It is evident only when the conidiophore is in the air, having a greenish opaque appearance. If the surface of the agar is flooded with water, the vacuole disappears, and though the water is removed immediately, the conidium is not discharged (fig. 21, 22). That this vacuole is not an optical illusion may be strikingly demonstrated by applying a 1 per cent solution of chromic acid to the surface of the agar. If this is done slowly, one may observe that the vacuole becomes discolored and may be forced up into the conidium by the addition of more and more acid. If the conidium is also flooded by the acid, the vacuole may be forced back into the stalk (fig. 35, a-d). It is of interest to note that the vacuole in the conidiophore stalk takes a distinctly different color with neutral red from those in the threads or conidia. In the conidiophore the vacuole becomes vellowish-orange with neutral red, while the other vacuoles are red.

Fig. 11-36.—Fig. 11. Semidiagrammatic surface and section view of small area of fungus on agar showing mycelium within agar, conidiophores some with conidia ready to be discharged, and others from which conidia have been ejected. Light comes from upper right. Note gametangia and two zygotes in agar also three conidia on surface of agar. X about 160.—Fig. 12-18. Successive stages in development and discharge of conidium. c, conidium; v, vacuole; vc, vacuole in conidium; cm, columella. Conidium (c) first appeared as a bud (fig. 12, c) 9:20 p.m. and was discharged fifty minutes later. Dotted line represents surface of agar. ×525.—Fig. 19. Mature conidium just after discharge. Note vacuole in papilla. ×525.—Fig. 20. Conidiophore just before and after discharge of conidium. Note vacuole in base of conidium in a and same vacuole in papilla in b. Conidium remained attached to columella. ×325.—Fig. 21. Conidiophore mounted in water showing strand of protoplasm flowing into conidium through tip of columella. Note large single vacuole has apparently been broken up into several smaller ones. ×930.—Fig. 22. Same conidiophore as above. After protoplasm had flowed into conidium, papilla evaginated, forcing columella to turn backwards into conidiophore. ×930.—Fig. 23, 24. Conidiophores germinating to form secondary conidia, the first with, the second without the stalk.  $\times 325$ .—Fig. 25, 26. Two conidia germinating on unsterilized egg albumen both of which have formed five secondary conidiophores. Vacuoles not shown. Small numbers, 1-5, in fig. 25 show order in which conidia were discharged.  $\times 525$ .—Fig. 27. Conidium with two basidialike conidiophores. On egg albumen. Note vacuoles. X335.—Fig. 28. Conidiophore with three secondary conidia. ×525.—Fig. 29. Conidium as shown in fig. 25, 26 after secondary conidia have been discharged showing six columellae with vacuole extending into each. On egg albumen. ×525.—Fig. 30. Conidiophore shortly before discharge of conidium showing increase in size of material in vacuole from a to b just before conidium was ejected. Note hyaline cytoplasm delimiting vacuole.  $\times 525$ .—Fig. 31. Same conidiophore as above showing collapse of vacuole and loss of rigidity of stalk.  $\times 525$ .—Fig. 32. Conidiophore stalk of *C. villosus* immediately after spore discharge. Opening in columella failed to close normally and vacuolar fluid was extruded in a drop as indicated by dotted circles. ×220.—Fig. 33. Conidiophore stalk of C. Brefeldianus immediately after discharge of conidium showing opening in columella which had apparently failed to close; apparent channel through vacuolar substance, and disintegrating vacuolated cytoplasm. ×525.—Fig. 34. Section of columella showing thickened wall. ×930.—Fig. 35. a, b, c, d. Diagrams showing how content of vacuole may be forced into conidium by adding chrom-acetic solution (Claussen's fluid) to surface of agar. Dotted lines indicate height of acid solution. As more acid was added, b, vacuole was forced up into conidium, c. When entire conidiophore (d) was submerged in acid by adding cover slip, vacuole now in two parts and with a greenish cast emerged from conidium back into stalk. ×525.—Fig. 36. a, b, c. Show diagrammatically how conidium throws itself off by sudden evagination of papilla. This apparently is caused by increased pressure in vacuole of conidium. The vacuole in stalk keeps conidiophore rigid. d shows what happens when vacuole in stalk is plasmolized by addition of film of water to surface of agar. The vacuole in conidium having no rigid opposing force now pushes the papilla and columella back into stalk, and conidium fails to throw itself off.  $\times$ 525.



After all or nearly all the protoplasm has passed into the conidium, the columella is formed. This begins to form on the side and progresses centrifugally, finally forming a dome-shaped structure which projects into the conidium. Sometimes the colummella begins to form before all the protoplasm enters the conidium. In figure 21 the columella is completely formed except for a pore at the top through which protoplasm is continuing to stream. After this long strand of protoplasm had passed in, the pore in the columella was closed. A few minutes after the columella is formed, another membrane is laid down over it. This will become the wall of the papilla of the conidium.

About ten minutes after all the protoplasm has passed into the conidium, it is shot away. Several minutes before this occurs, a dome-shaped vacuole is formed over the columella (fig. 17, 20a). This vacuole increases in size, doubtless due to the absorption of liquid through the conidiophore (fig. 36a, b, c). The strong pressure within the spore created by this vacuole counterbalanced by the vacuole in the stalk (one can easily observe the increase in size of the vacuole in the stalk just before the conidium is discharged) brings about the annular rupture of the wall of the spore at the base of the columella. The instant this rupture occurs the papilla reverses its position with such violence that the spore is hurled through the air for 5 to 12 mm. (fig. 10). My observations agree essentially with those of Gallaud (1905) and Martin (1925), though neither of these authors emphasized sufficiently the rôle played by the columella, stalk, and vacuole in spore discharge. Before the spore is discharged, the columella wall becomes much thickened, and this reinforced wall normally prevents the columella from being pushed back into the stalk. Indeed, if a conidiophore is flooded with water shortly before the spore is ready to be discharged. the pressure within the spore may become so great that the columella is forced back into the stalk (fig. 22; see also Gallaud, 1905). The vacuole within the stalk plays an equally important rôle in that it furnishes the turgor pressure to keep the conidiophore rigid and thus furnishes the conidium an opposing force against which it may kick itself off.

If a culture is left upright on the table with uniform illumination from all sides, the conidia are shot out irregularly in all directions. But if a culture is illuminated more strongly on one side or, better, if it is put in a black box or other dark container with a small hole on one side to permit the entrance of light, the conidiophores will point toward the source of light, and the conidia will be shot in that direction (fig. 3, 4, 10, 11). The conidia may be hurled horizontally a maximum distance of 12 mm. or vertically 8 mm. (fig. 10).

If conditions are unfavorable for germination, a conidium may form a secondary conidium usually without a stalk (fig. 23, 24). This secondary conidium is shot away in the same manner toward the source of light. A third one may form and be shot away and so on until the conidium germinates or dies. By such behavior the protoplasm in one and the same conidium may travel across a plain agar plate (i.e., agar without nutrients) in a few hours' time.

Sexual reproduction.—The present fungus is capable of forming zygospores on a variety of culture media. The most favorable solid culture medium of a large number tried was agar No. 35 (1 liter  $\rm H_2O$ , 20 grams agar, 3 grams maltose, 4 grams meat peptone) on which the production of zygospores was very abundant, the number varying between 200 and 300 in the lower power field of view. A reduction or an increase in this amount of peptone caused a decrease in the production of zygospores. A very few zygospores are formed on plain agar.

Fig. 37-53.—Fig. 37. Threads on agar culture (agar no. 5) showing empty segments, segments with cytoplasm (note vacuoles), and lateral and scalariform conjugation. ×213.—Fig. 38. Male and female gametangia conjugating. In figure to right male cell resembling antheridium has been cut off; in figure to left male cell has emptied into larger. ×213.—Fig. 39. Thread showing young zygote below and gametangia about ready to conjugate above. Note how empty part of male segment is separated from protoplast as the latter moves toward female cell. ×213.—Fig. 40-46. Stages in conjugation of gametangia.—Fig. 40. 11:30 a.m., May 23. Both gametangia have reached mature size, and the protoplasm is flowing from the suspensors into the gametangia. ×787.—Fig. 41. 11:35 a.m., May 23. Smaller gametangium has been separated from suspensor by cross wall which is now straight in section view. Note vacuole in smaller gametangium. ×787.—Fig. 42. 11:40 a.m., May 23. Basal wall of smaller gametangium has become convex outwards, indicating internal pressure. Note vacuole and concentration of hyaline cytoplasm on wall between two gametangia. ×787.—Fig. 43. 11:41-11:47 a.m., May 23. Protoplasm of smaller gametangium began flowing into larger about 11:40, and by 11:47 the smaller was entirely empty. The dotted lines represent the successive positions of the plasma membrane at intervals of slightly more than a minute. ×787.-Fig. 44. 11:48 a.m., May 23. Suspensor of larger gametangium has become empty, and wall has formed, separating larger gametangium from its suspensor. For a few minutes after the smaller gametangium had emptied, a minute pore was visible in the wall between the two gametangia. By 12:20 the smaller gametangium had begun to collapse. ×787.—Fig. 45. One day later. Zygote wall thickening, now two layered. Oil globules coalescing to form larger globules. ×787.-Fig. 46. Two days later, zygote nearly mature. Wall apparently three layered, middle layer thickest. Note large oil body. ×787.—Fig. 47-50. Stages in maturation of zygote. ×787.—Fig. 51. Showing two gametangia from different threads. ×787.—Fig. 52. Nearly mature zygote showing pore in wall through which smaller gametangium emptied. X1395.-Fig. 53. Zygote several weeks old. Note eccentric oil globule as in Achlya sp. Wall consisting of three layers: (1) thin inner greenish layer; (2) thick middle layer; (3) thin outer layer, perhaps old gametangial wall. ×1500.

Table 1. Summary of characteristics of genera of Entomophthorales.

		ENT	ENTOMOPHTHORACEAE				BASIDIOBOLACEAE
Delacroixia	Conidiobolus	Entomophthora	Empusa	Massospora	Completoria	Ancylistes	Basidiobolus
Saprophytic mostly.	Saprophytic mostly. Saprophytic mostly. Parasitic, genous, 30 cultivated cultivated phyte.	Parasitic, entomogenous, 30 species, 3 cultivated as saprophyte.	Parasitic, ento-mogenous, 11 species, 1 cultured as saprophyte.	Parasitic, ento- mogenous.	Parasitic on fern prothallia.	Parasitic on desmids.	Saprophytic on frog dung but culturable on wide variety of substrata.
Hyphae well developed with multinucleate segments. No cellulose.	Hyphae well developed, with multinucleate segments. No cellulose.	Mycelium well developed. Segments with few nuclei (so far as known). No cellulose (?).	Mycelium well developed. Segments multinucleate (so far as known). No cellulose.	o.	Mycelium poorly developed.	Mycelium of limited growth. Segments multinucleate. No cellulose.	Mycelium well developed. Segments uninucleate. Cellulose present.
Conidiophore un- branched, with per- sistent conical colu- mella.	Conidiophore un- branched, with dis- tinct and persistent conical columella.	Conidiophore branched.	Conidiophore un- branched.	Conidiophore un- branched.	Conidiophore un- branched.	Conidiophore un- branched, with conical columella.	Conidiophore branched.
Conidia spherical or oval with apiculus, forcibly discharged.	Conidia spherical or oval with apiculus, forcibly discharged.	Conidia uninucleate (so far as known), mostly elongate.	Conidia multinu- cleate, spherical or broadly ovoid.	Conidia oval with thick, rough wall. Formed in loc- ules.	Conidia spheri- cal, forcibly dis- charged.	Conidia spheri- cal, forcibly dis- charged, as in Conidiobolus.	Conidia spherical or oval, forcibly discharged but not as in Conidiobolus.
Zygospores unknown, perhaps supplanted by villose spores developed from conidia.	Zygospores unknown, Zygospore produced Zygospore or azygoperhaps supplanted in one of the conspores formed by by villose spores dejugating cells—budding, the bud veloped from collamost filling galaxies arising from one or metangial wall. Oil both gametangia, globule eccentric. Oil globule centric or eccentric.		Azygospore formed by budding, the bud arising from a hyphal body. Oil globule centric or eccentric.	Azygospore formed by budding. Oil globule (?).	Azygospore formed. Oil globule (?).	Zygospore produced in one of conjugating cells. Lying free in female cell. Oil globule centric.	Zygospore produced in one of conjugating cells. Oil globule (?).

The maximum zygospore formation in liquid solutions occurred when cultures were grown in 0.25 per cent peptone plus 2 per cent malt extract for three days, upon which this fluid was replaced by 0.25 per cent peptone alone for two days, and then the cultures were washed and left in water. In such cultures sexual reproduction was exceedingly abundant, thus differing from cultures in which the nutrient fluids were not changed. Similar results have been obtained by Klebs, Kauffman, and others with Saprolegnia and Achlya.

While light favors the formation of conidia, the zygotes are most abundant in cultures kept in total

darkness.

The development of the zygote may be divided into three rather distinct stages; first, the formation and maturation of the gametangia; second, the union of the two protoplasts; and third, the ripening of the zygote. Conjugation usually occurs between gametangia in the same filament but may also occur between gametangia which arise from different filaments (fig. 37, 51). Segments between which conjugation occurs are in contact before the gametangia begin to swell; this is quite obvious where the conjugation is lateral but not so easy to demonstrate when conjugation is scalariform. The first indication that conjugation is going to occur between two segments is the swelling of the adjacent ends. The time from the first swelling until the gametangia are mature is about six hours. One of the gametangia, the female, swells until it becomes a spherical body about four times the diameter of the thread that bears it, while the male swells to about twice the diameter of its supporting thread. Sometimes the male suspensor empties directly into the female gametangium without the formation of a swollen male gametangium. After the two gametangia have reached mature size, the protoplasm in the suspensors flows into the gametangia (fig. 40). Usually, however, the male suspensor becomes empty before the female. Although the two conjugating segments are of about equal size at first, the female apparently contains a much larger amount of protoplasm. As a rule when the protoplasm has completely passed from the suspensor into the smaller gametangium, a wall is laid down separating the suspensor from the gametangium, and thus this structure resembles the antheridium of some species of Lagenidium or Pythium (fig. 41). This wall, at first plane, very shortly bulges outward, indicating increased internal pressure within the smaller gametangium (fig. 42). Vacuoles appear in the latter, and soon the protoplasm begins to empty from the smaller into the larger, this latter process occupying only five to ten minutes (fig. 43). For a few minutes after the smaller gametangium has emptied, a distinct pore is visible. Three to five minutes later the protoplasm in the female suspensor moves into the larger gametangium, after which it is separated from the suspensor by a wall (fig. 44). Usually after the smaller gametangium has emptied, its wall partially collapses (fig. 45,

46), but, since the wall is considerably thickened, it may remain visible for months attached to the avente.

After the two protoplasts have united, the zygote begins to mature. The wall becomes greatly thickened, after two to three days finally consisting of three layers, an outer thin, a middle thick, and an inner thin layer (fig. 46, 52, 53). It must be admitted, however, that though the wall was studied with a Zeiss water-immersion lens and oculars 15× and 20× and with an excellent source of illumination (Spencer lamp with prism), it was impossible to be certain about the inner thin wall layer. Both Thaxter (1888) and Riddle (1906) in their studies on the Entomophthoraceae showed zygotes with a two-layered wall.

As the zygote ripens, the small oil (?) bodies coalesce until finally one large globule is formed. In the thoroughly mature zygote this globule is eccentric, the structure strikingly resembling that of an eccentric egg in Achlya (fig. 53). The two may easily be distinguished, for in Achlya the protoplasm of the egg contains large dark granules, while the protoplasm in the zygote of Conidiobolus has a

whitish gleam.

Germination of zygote.—After the zygote has rested for about two weeks, it is capable of germination. It may retain its vitality for at least six months, perhaps longer. Zygotes dried in agar for six months were still capable of germination. The detailed stages in germination have not been followed, but it has been observed that during germination the oil globule disappears and the whitish protoplasm becomes coarsely granular. The zygote may then form a hypha which grows into a new mycelium or it may produce one conidiophore.

Contrasts with other fungi.—In view of the success of Blakeslee in securing imperfect hybridization between different species of mucors, it seemed of great interest to try such reactions between Conidiobolus Brefeldianus and other supposedly related forms. Accordingly the following contrasts have been made on agars No. 35 and 41, on both of which many zygotes are formed in C. Brefeldianus and very few conidia: C. Brefeldianus X C. villosus (Chapel Hill strain); C. Brefeldianus X C. villosus (Cornell strain from White); C. villosus (Cornell) X C. villosus (Chapel Hill); C. Brefeldianus X Basidiobolus ranarum (from Maneval); C. villosus (Cornell) × Basidiobolus ranarum (from Maneval); C. villosus (Chapel Hill) × Basidiobolus ranarum (from Maneval). In all cases the results were negative.

Comparison of Conidiobolus with other genera of the Entomophthoraceae.—The writer is following the suggestion first made by Raciborski (1896) and later followed by Claussen (1924) and Gaümann (1926) and excluding Basidiobolus from the Entomophthoraceae, placing this in a family of its own, the Basidiobolaceae. This action is fully justified by the researches of Fairchild (1897), Raciborski (1896), Olive (1907), Levisohn (1927), and others on Basidiobolus, which need not be reviewed here, and, as a further indication that this genus should be separated, the writer has found that the cell wall of Basidiobolus contains cellulose as indicated by a beautiful deep pinkish-purple reaction with chloroiodide of zinc, while species of Conidiobolus, Ancylistes, and Empusa which have been tested do not give such a reaction. From the above it is obvious that the relationship of Conidiobolus to Basidiobolus is remote.

The fungus known in this country as Conidiobolus villosus Martin must be compared with the genus as represented by C. utriculosus and C. Bre-This fungus was first described by Costantin (1897) as Boudierella coronata, renamed Delacroixia coronata by Saccardo and Sydow (1899), and has since been collected and studied by Gallaud (1905), Gilbert (1919), Martin (1925) (who named the fungus Conidiobolus villosus), White (1937), and Kevorkian (1937). The writer has also isolated and now has in culture the same species from the old sporophyte stalks of Nardia where it was growing as a weak, endophytic parasite. The fungus has been regarded as a saprophyte or perhaps a weak parasite. In his recent paper Kevorkian (1937) reports studies on the same fungus from living termites and, because of the morphological similarities between the forms studied by Costantin, Gallaud, Gilbert, Martin, and himself, has reduced all of these to synonomy. Furthermore, in view of the ability of the saprophytic Delacroixia coronata to parasitize termites and the ability of certain parasitic species of Empusa (Brefeld, 1884; Sawyer, 1929; and others) to live as saprophytes, Kevorkian places the species Delacroixia coronata (Cost.) in the genus Entomophthora. The writer agrees with Kevorkian's first conclusion but thinks it inadvisable to reduce the genus Delacroixia to synonomy with any other genus of the Entomophthoraceae until the zygospores of the former have been found. The genus Delacroixia is very close to Conidiobolus (see Gallaud, 1905; Martin, 1925); indeed, until the zygospores of Delacroixia are discovered, the villose resting spores of the latter must serve as the sole means of distinguishing the two. Likewise Delacroixia may be distinguished from Empusa (see table 1) by the villose resting spores.

Before a comparison can be made between Conidiobolus on the one hand and Entomophthora and Empusa on the other, it is necessary to discuss briefly the status of the two last-named genera. The writer feels that nothing is to be gained by combining Entomophthora and Empusa into one genus (Thaxter, 1888) but prefers to follow Nowakowski (1883) and other European students of this group in recognizing both genera (see also Sawyer, 1929). In table 1 the more important characters of the genera of the Entomophthoraceae are given. It may be seen that at present the chief differences between the two genera Empusa and Entomophthora are that in Entomophthora the co-

nidiophores are branched and the conidia are uninucleate, while in Empusa the conidiophores are unbranched and the conidia are multinucleate. Another important difference in the two genera which has apparently been overlooked by others but which shows up strikingly in Thaxter's figures has to do with the shape of the conidia. In Empusa the primary conidia are mostly globose, while in Entomophthora the primary conidia are elongate. A careful study of the structure of the conidiophore and the mode of conidial discharge is urgently needed in both genera. According to the cytological researches of Olive (1906) there are at least two methods of spore ejection in the six species of Empusa (including Entomorphthora sp.) investigated by him.

From the table and the above discussion it is obvious that Empusa with its multinucleate mycelial segments, unbranched conidiophores, spherical or broadly ovoid, multinucleate conidia, is closer to Conidiobolus than is Entomophthora with its branched conidiophores and elongate, uninucleate conidia. It will suffice, therefore, to differentiate between Empusa and Conidiobolus. As pointed out by Thaxter (1888), Empusa may be separated from Conidiobolus by the fact that in Empusa (and Entomophthora) we have a zygospore or azygospore formed by budding, while in Conidiobolus the spore is produced directly within one of the conjugating cells (see also Riddle, 1906; Nowakowski, 1877). This difference in the formation of the zygospore is of fundamental importance and should be emphasized as the main generic distinction between Conidiobolus and Empusa. Even if we are forced to abandon the physiological distinction between Conidiobolus and the entomogenous forms, this striking morphological distinction will still be of generic importance. It seems to the writer, however, that before this physiological distinction can be abandoned, much more work needs to be done, for of the forty or more species of Entomophthora and Empusa only four have yet been isolated in culture (Sawyer, 1929, p. 90 et seq.).

The remaining entomogenous genus, Massospora (Spear, 1921), with its spores borne in locules as in certain Gasteromycetes (Goldstein, 1929), is so distinct from any of the other genera that comparison is superfluous.

The two remaining genera Completoria (Lohde, 1874; Leitgeb, 1881; Atkinson, 1894) and Ancylistes (Berdan, 1937, 1938) are obligate parasites on plants, the former in fern prothallia, the latter in desmids. So far no one has been successful in culturing either of these as saprophytes. Miss Berdan, in our laboratory, tried growing Conidiobolus villosus and C. Brefeldianus on fern prothallia, but without success. From a morphological standpoint not enough is known of the conidia and the mode of formation of the resting spore (?) in Completoria to justify comparison with Conidiobolus. The genus Ancylistes, our knowledge of which has recently been greatly extended by Miss Berdan,

shows some rather striking affinities with Conidiobolus. The conidiophores, the mode of discharge of the conidia, and the appearance of the columella and stalk after discharge are strikingly alike in both genera. In sexual reproduction, however, Ancylistes is distinct from all other genera in this order in that in Ancylistes (except perhaps for A. Pfeifferi) the contents of the male cell or gametangium pass into the female gametangium through a tube which arises from the male cell (Pfitzer, 1872; Dangeard, 1906; Berdan, 1938). In Ancylistes the zygote retracts from the wall more than in either Conidiobolus utriculosus or C. Brefeldianus. Indeed, in Ancylistes Pfeifferi (Berdan, 1938) this retraction is such that the zygote in the old mother cell wall, which is composed largely of blunt protuberances, resembles the ripe oogonia of Achlya glomerata in a most striking way. It is obvious, therefore, that though Conidiobolus and Ancylistes are closely related, they should remain distinct.

Finally the question arises: Is the sexual process in Conidiobolus and Ancylistes oomycetous or zygomycetous? Brefeld (1884) considered the sexual process in Conidiobolus distinctly comycetous, and in C. utriculosus and the present species the smaller gametangium resembles an antheridium, but the larger gametangium only superficially resembles an oogonium for the simple reason that no egg is differentiated in this structure. Even in Ancylistes, where the resemblance of the sexual organs to those of the oomycetes is so marked that this has been the only reason for placing the genus with Lagenidium (Pfitzer [1872] noted the absence of cellulose as indicated by the chloroiodide of zinc reaction), the comvectous appearance is of no phylogenetic significance, for no egg is differentiated in the female structure, and the structure which resembles an egg is the zygote. Another peculiarity in zygote formation in Ancylistes and Conidiobolus is the cutting off of one or several empty cells from the female cell (Pfitzer, 1872). This usually occurs after plasmogamy but may occur while the male protoplast is flowing into the female cell. Thus, the zygote is formed not in the cell in which plasmogamy occurred but only in part of it. Moreover, the nuclear behavior during sexual reproduction of Conidiobolus agrees with that described by Olive (1906) and Riddle (1906) for Entomophthora in that both gametangia are multinucleate, but nuclear fusion, if it occurs in Conidiobolus, must take place during zygote germination.

#### SUMMARY

Conidiobolus Brefeldianus n. sp., characterized by its delicate growth, small conidia, and small zygospores, is described.

The fungus is culturable on a great variety of plant and animal substrata and is capable of para-

sitizing certain insects.

The formation of conidia is determined by proper aeration, favorable nutrients, and light. On favorable nutrient material conidia are formed in continuous darkness or continuous light. The number of conidia is greatly increased on any favorable culture media if the culture is exposed to alternating daylight and darkness.

Conidial formation is very rapid, the whole process taking from fifty minutes to two hours. When mature, the conidium hurls itself horizontally for 5 to 12 mm. by the sudden outpushing of the papilla. This force acts against the cone-shaped columella. The turgor of the conidiophore is maintained by a conspicuous vacuole, and if the latter is plasmolized, the conidium fails to get away.

Sexual reproduction occurs on a great variety of culture media but is most abundant in media relatively rich in peptone. Conjugation is usually lateral but may occur between two gametangia from different threads. The gametangia are of unequal size, the smaller always emptying into the larger to form the zygote. Both gametangia are multinucleate, as are also the zygotes. Nuclear fusion has not yet been observed. The ripe zygote has a large eccentric fat (?) globule and whitish cytoplasm surrounded by a three-layered (?) wall.

The zygote may germinate after about two weeks' rest but will retain its vitality for at least six months.

Contrasts with two strains of *Delacroixia coro*nata as well as with *Basidiobolus ranarum* produced no imperfect hybridization.

Conidiobolus is compared with the other genera of Entomophthoraceae and because of its saprophytic habit and method of zygospore formation is retained as a valid genus.

Basidibolus is shown to have cellulose walls by the chloroiodide of zinc reaction, thus verifying its position in a separate family.

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# EVIDENCE FOR THE PRESENCE IN CERTAIN ETHER EXTRACTS OF SUBSTANCES PARTIALLY MASKING THE ACTIVITY OF AUXIN <sup>1</sup>

#### Richard H. Goodwin

Positive curvatures<sup>2</sup> of Avena coleoptiles upon inilateral application of various plant extracts in igar have been reported by Stark (1921), Nielsen (1924), Seubert (1925), Czaja (1934), Höfner (1937), Stewart, Bergren, and Redemann (1939), and others. The writer also has obtained positive urvatures ranging between 6° and 19° (d values 1.16 to 0.58) from ether extracts of Taraxacum oots while testing for the auxin content by the Avena technique of Boysen Jensen (1937). These indings suggest that substances other than auxin any frequently be present in extracts and that they have a toxic or inhibiting effect upon the growth f the Avena coleoptile. In testing extracts for their uxin content by the Avena method, the presence of

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<sup>2</sup> Curvatures toward the applied agar block as conasted with the negative curvatures normally obtained ith suitable concentrations of growth-promoting subances. such substances would tend to counteract or mask the activity of the auxin, thereby introducing errors in quantitative determinations.

The purpose of this investigation is to throw further light upon the nature of ether extracts from corn meal and *Vicia Faba* shoots. Experiments were planned to ascertain whether or not masking substances were present in these extracts. The likelihood of the masking substances having the same molecular weight and hence the same diffusion coefficient as auxin would be small. Therefore, determinations of the diffusion coefficients of the auxin in the extracts were made as described below. These were compared with the theoretical values for auxin a and auxin b, natural growth-promoting substances found in higher plants.

Diffusion coefficients were determined by the method of Graham (1861) as applied to substances influencing cell elongation in Avena by Went (1928) and Heyn (1935). The method as used in the present investigation consisted in allowing the active substances present in discs of 1.5 per cent agar, 1 mm. thick, and 100 mm.<sup>3</sup> in volume, to diffuse at 20°C. into stacks of three other similar discs of pure agar. After a given time the four discs were

separated, and 12 blocks 4 mm.<sup>3</sup> were cut from each. The activity of the substances contained in the blocks was determined by the Avena test of Boysen Jensen (1937). Exact details of the test conditions in this investigation have been described by Goodwin (1939). From these data the percentage of active substances present in each of the four discs was calculated. Kawalki (1894) gives a table (modified from Stefan, 1879) of the theoretical percentages of any pure non-electrolyte in each of

Table 1. Diffusion of 3-indole acetic acid through 4 discs of agar.

		3-indole	2			
		acetic				
		acid,	% in			
No. of		$\gamma$ per	each		Ave.	
disc	$d^{\mathbf{a}}$	literb	disc	æ	x	D
1	1.22	81	33.5	0.099)		
2	1.15	73	30.0	0.130	0.700	0.550
3	0.93	52	21.5	0.098	0.109	0.550
4	0.66	37	15.1	0.101		
		243				
1	1.20	80	33.3	0.097)		
2	1.12	70	28.9	0.109		
3	0.92	51	21.2	0.103	0.102	0.588
4	0.73	40	16.7	0.099		
		$\overline{241}$				
1	1.26	85	34.8	0.1097		
2	1.16	74	30.1	0.135	0 770	
3	0.95	53	21.7	0.094	0.116	0.517
4	0.56	33	13.3	0.125		
		245				
1	1.39	101	37.6	0.130)		
2	1.18	77	28.4	0.099	0.705	0.400
3	0.86	47	17.5	0.171	0.125	0.480
4	0.82	45	16.5	0.099		
		270				
Average		250			0.113	0.531
Theoretic	al	240			0.114	0.529

<sup>\*</sup> d = the difference of length in mm. between the convex and concave sides of the coleoptile.

four equal layers after diffusion, for various values of the expression  $h^2/4Dt$  (or x), where h is the thickness of each layer in cm., t is the time in days, and D is the diffusion coefficient of the non-electrolyte. By means of this table the corresponding values of x for the empirical percentages obtained in each diffusion experiment were determined by linear interpolation.

The accuracy of the method was first tested with 3-indole acetic acid (molecular weight (M) = 175; diffusion coefficient at 20°C.3 = 0.529). Discs of 1.5 per cent agar containing 0.24 mg. of 3-indole acetic acid per liter were diffused for one hour, under which conditions  $h^2/4$   $t = 0.1^2/4$  (1/24) =0.06, and the theoretical value of x is 0.1135. The results are given in table 1. A pronounced gradient was obtained in each case (columns 3 and 4), and the average diffusion coefficient is very close to the theoretical value. Since the coefficient of variability of the averages of the diffusion experiments is not much larger than that of direct tests as shown in table 2, the variability of the results may be attributed in good part to the test method. The errors introduced by the diffusion technique are not large.

Determinations of the diffusion coefficients of auxin in ether extracts of plant material were then made. Stock auxin extracts were prepared from corn meal by the following method, recommended by Boysen Jensen: 200 g. of coarse corn meal, freshly-ground from dormant kernels, were covered with 400 cc. of peroxide-free ether (1 liter of ether shaken with 0.5 g. CaO + 2.5 g. FeSO<sub>4</sub> + 10 cc. water and distilled) for about 48 hours and shaken occasionally. The ether extract was then drawn off, filtered, and evaporated to about 30 cc. In order to separate the auxin from excessive fats, the extract was shaken in a separating funnel with two changes of basic, saturated glucose solution (25 cc. glucose solution + 1 cc. 8 per cent NaHCO<sub>3</sub>) for five minutes each. The basic ether fraction was then discarded. The glucose solution was acidified with 3 cc. of 10 per cent tartaric acid and shaken with three successive changes of 35 cc. of fresh ether for

 $^3$  D<sub>20°C.</sub> =  $7/\sqrt{M}$  =  $7/\sqrt{175}$  = 0.529 (see öholm, 1910).

	Diffusion experime	Direct tests (data from Goodwin [1939] table 2, columns 1 and 3)			
No. of	3-indole acetic acid, $\gamma$ per liter, theoretical value	3-indole acetic acid, γ per liter, average obtained	Coefficient of variability of the average	3-indole acetic acid, γ per liter	Coefficient of variability of the average
1	85	$87 \pm 4.9^{a}$	5.6	120	3.5
2	70	$74 \pm 1.5^{a}$	2.0	80	2.2
3	50	$51 \pm 1.3$ *	2.6	60	2.3
4	35	$38 \pm 2.6^{a}$	6.8	40	4.9
- <del>-</del>				20	17.5

Table 2. Comparison of the variability of the diffusion experiments and direct tests.

<sup>&</sup>lt;sup>b</sup> Values calculated from an activity-concentration curve for 3-indole acetic acid (Goodwin [1939], fig. 1, curve A).  $1\gamma = 0.001$  mg.

a Standard deviation of the average.

Table 3. Diffusion of auxin extracted from corn meal through discs of agar.

No. of	No. of			diffusion			Second	diffusion	
extract	disc	d	WAE × 10 <sup>-3a</sup>	% in each disc	$\boldsymbol{v}$	d	WAE	% in each disc	. w
1	$\begin{cases} 1\\2\\3\\4 \end{cases}$ Average	0.81 1.03 0.78 0.66	0.82 1.03 0.79 0.69	25.6 31.0 23.8 20.7	0.035 0.163 0.060 0.068 0.082	1.01 0.83 0.61 0.30	1.01 0.83 0.65 0.45	34.5 28.1 22.2 15.2	0.10 0.08 0.08 0.10
2	1 2 3 4 Average	1.10 1.34 1.14 1.01	1.10 1.43 1.15 1.01	23.5 30.6 24.6 21.6	0.000 0.149 0.043 0.062 0.064	1.39 1.24 0.90 0.77	1.50 1.28 0.90 0.78	33.6 28.7 20.2 17.5	0.09° 0.10° 0.10° 0.12° 0.09°2
3	$\begin{cases} 1\\2\\3\\4 \end{cases}$	1.09 1.10 0.97 0.95	1.09 1.10 0.97 0.95	26.5 26.8 23.6 23.1	0.046 0.071 0.063 0.050 0.056	1.60 1.40 1.10 0.89	1.95 1.52 1.10 0.89	35.7 27.8 20.2 16.3	0.104 0.115 0.088 0.120 0.101
4	$\begin{cases} 1\\2\\3\\4\\ \text{Average} \end{cases}$	1.28 1.26 0.98 0.44	1.33 1.30 0.98 0.54	32.1 31.4 23.6 13.0	0.089 0.181 0.063 0.128	1.38 1.24 0.86 0.54	1.48 1.28 0.86 0.60	35.0 30.3 20.4 14.2	0.106 0.120 0.142 0.116 0.117 0.124
4	$egin{cases} 2 \\ 3 \\ 4 \\ Average \end{cases}$					1.13 0.91 0.53 0.23	1.14 0.91 0.59 0.42	37.3 30.0 19.3 13.7	0.124 0.128 0.125 0.136 0.122

<sup>&</sup>lt;sup>a</sup> For a definition of WAE see footnote 4. WAE were calculated from an activity-concentration curve for auxin from corn meal (the mean of 3 extracts). Each value should be multiplied by 10<sup>-3</sup>.

Table 4. Summary of tables 1 and 3.

	Average $x = h^2/4Dt$	Diffusion coefficient $(D)$			
Extract from corn	First Second diffusion diffusion	First diffusion	Second diffusion		
(values from table 3)  Average  Theoretical values for auxin b Theoretical values for auxin a	$\begin{array}{ccc} 0.082 & 0.097 \\ 0.064 & 0.104 \\ 0.056 & 0.106 \\ 0.115 & 0.126 \\ \hline 0.079 \pm 0.013^a & \hline 0.108 \pm 0.006^a \\ 0.124 & & & & & & & & \\ \end{array}$	$0.585$ $0.750$ $0.857$ $0.417$ $0.652 \pm 0.096^{a}$ $0.398$ $0.387$	$0.495 \\ 0.461 \\ 0.453 \\ 0.381 \\ \hline 0.448 \pm 0.024$		
3-indole acetic acid (values from table 1)  Average	$0.109 \\ 0.102 \\ 0.116 \\ 0.125 \\ \hline 0.113 \pm 0.005^{a}$	$0.550 \\ 0.588 \\ 0.517 \\ 0.480 \\ \hline 0.531 \pm$	- 0.0234		
3-indole acetic acid  a Standard deviation of the me	0.114	0.529			

<sup>&</sup>lt;sup>a</sup> Standard deviation of the mean.

five minutes each. The glucose was then discarded. The acid-ether fraction was evaporated to about 1 cc. and the residue made up to 25 cc. of chloroform.

Measured amounts of these stock solutions were diluted from time to time and suitable quantities evaporated to dryness, taken up in 0.8 cc. of ether and slowly dropped onto pure 1.5 per cent agar discs, where the ether was evaporated according to the micro-method of Boysen Jensen (1937). Each agar disc was composed of two layers, the bottom one 1 mm. and the top one 0.25 mm. thick. The top layer along with any relatively water-insoluble scum was removed about an hour after the ether was evaporated.

The assumption was made that the auxin in the extracts had a larger molecular weight than 3-indole acetic acid. Hence, diffusions of the extracts within the stacks of four discs were carried out for 1.25 hours, a slightly longer period of time than in the previous experiments. In this case  $h^2/4t =$ 0.048 and a suitable gradient should be obtained with auxin a (M = 328) or auxin b (M = 310). The results of diffusion experiments with four different extracts are given in table 3, columns 2 to 5, and are summarized in table 4. It can be seen that in each first diffusion  $x = h^2/4Dt$  is much too low and the diffusion coefficient (D) too high for auxin a or b. When about five times as much of the same extracts was dropped originally and was diffused for 1.25 hours as usual, and the bottom discs of each stack were then diffused for another 1.25 hours, each one on three more discs of pure agar, the results of these second diffusions, shown in table 3, columns 7 to 10, and summarized in table 4, are in each case significantly closer to the theoretical values for auxin a and b. Since the diffusion coefficient of a single pure substance should, under comparable conditions, be constant, we may conclude that we are not dealing with a single substance in these extracts.

It should be pointed out that standard deviations of x and D for 3-indole acetic acid and for second diffusions of corn meal extracts are of the same magnitude (see table 4). This indicates that no further errors have been introduced.

These results may find an explanation in the following hypothesis-namely, that in addition to auxin, substances which have a molecular weight greater than auxin and which mask the effect of the auxin either by directly inhibiting cell elongation or by inactivating or otherwise reducing the growthpromoting effect of the auxin, are present in the extracts. These substances of larger molecular weight should diffuse more slowly than auxin, their relative concentration and hence their masking effect should be greatest in the first disc, and the gradient determined should be smaller than the correct value for auxin. Note in table 3, column 5, that the percentage of activity in the first disc is consistently low. Table 5 shows these low percentages to be experimentally significant. In second diffusions

Table 5. Data from tables 1 and 3.

Theoreti- cal per-	Theoreti-	
of 3-indol	cal per- e centage	Observed mean per-
	a of auxin $a$ in the	centage of activity in
first disc	first disc	the first disc
First diffusion	36.8	26.9 ± 1.8ª
Second diffusion	36.8	$35.0 \pm 0.6^{a}$
3-indole acetic acid 35.5		$34.8 \pm 1.0^{a}$

a Standard deviation of the mean.

there should be much less of the masking substances present, and hence the diffusion coefficients should be nearer the theoretical value for auxin.

The following alternative explanations do not appear to be feasible. If a masking substance of lower molecular weight than auxin were present, relatively higher percentages of the substance should be obtained in the fourth disc after diffusion, resulting in a steeper gradient and hence a smaller diffusion coefficient than that expected for auxin. This effect would be accentuated in the second diffusion. If, on the other hand, there were two or more active substances differing in molecular weight, there would be a relatively higher percentage of substances with lower molecular weights and larger diffusion coefficients in the fourth than in the first disc after the first diffusion and hence, a larger empirical value for D in the second than in the first diffusion.

If masking substances are present in an extract in appreciable amounts, accurate quantitative determinations of the auxin by means of the Avena test cannot be made directly. To illustrate this point, the amount of auxin in WAE<sup>4</sup> per kilogram of corn meal in each of the four corn meal extracts was calculated by three different methods: first, from direct tests of the solution by the method of Boysen Jensen (1937); second, from an average of the values obtained in the first diffusion; and third, from an average of the values obtained in the second diffusion (assuming that 13.4 per cent of the auxin—the theoretical amount for auxin a—reaches

Table 6. The amount of auxin in WAE per kilogram of corn meal as calculated by three methods.

Direct test	Average of first diffusion	Average of second diffusion		
4.2	3.5	6.0		
8.8	9.9	19.6		
	6.3	15.0		
2.7	4.9	4.7		

<sup>&</sup>lt;sup>4</sup> One WAE is the amount of auxin dissolved in 100 cc. of 1.5 per cent agar which, when applied unilaterally to Avena coleoptiles in 4 mm.<sup>3</sup> blocks at a temperature of 21 to 22°C., will produce in three hours a curvature with a d value of 1 mm.

Table 7. Diffusion of auxin extracts through four discs of agar.

Material extracted No.	of disc	d	WAE × 10⁻³⁵	% in each disc	$\boldsymbol{x}$	Ave. x
Maize endosperm	1	1.19	1.22	28.4	0.063)	
	2	1.18	1.20	27.9	0.090	0.069
	3	1.06	1.06	24.6	0.044	0.009
	4	0.81	0.82	19.1	0.080	
Maize embryos	1	0.70	0.72	29.2	0.068)	
	2	0.66	0.69	27.9	0.089	0.000
	3	0.42	0.53	21.5	0.098	0.080
	4	0.42	0.52	21.1	0.065	
Vicia Faba, light-grown seedlingsa	1	1.11	1.11	26.1	0.042)	
, 5	2	1.08	1.08	25.4	0.040	
	3	1.08	1.08	25.4	0.000	0.033
	4	0.98	0.98	23.1	0.050	
Vicia Faba, light-grown seedlingsa	1	1.38	1.49	36.2	0.119)	
	2	1.08	1.08	25.4	0.040	0.100
	3	0.91	0.91	22.1	0.086	0.109
	4	0.44	0.54	13.1	0.127	
Vicia Faba, light-grown seedlingsa	1	0.39	0.51	26.0	0.041)	
	2	0.54	0.60	30.6	0.149	
	3	0.39	0.51	26.0	0.000	0.071
	4	0.10	0.34	17.4	0.092	
mltill for one in h		-				0.121
Theoretical value for auxin $b$ Theoretical value for auxin $a$						0.121

<sup>&</sup>lt;sup>a</sup> The apical 6 cm. of the shoots were ground in five changes of ether which had been chilled with solid carbon dioxide. The ether extracts were tested immediately without purification.

<sup>b</sup> Each value should be multiplied by 10<sup>-3</sup>.

the bottom block by the end of the first diffusion). The results are shown in table 6. With the exception of the last extract which appears to be relatively pure, calculations from second diffusions are about twice as large as those from first diffusions. Since second diffusions were all carried out on the day following the first diffusions, loss of activity during this time may have reduced the true magnitude of the discrepancy.

The diffusion coefficients of a few more extracts are shown in table 7. Masking substances appear to be present in extracts of both endosperm and embryos of maize and also in extracts of green *Vicia Faba* shoots.

#### SUMMARY

Determinations of the diffusion coefficient of auxin in ether extracts of corn meal and Vicia Faba

shoots were made. The values obtained did not agree with the theoretical diffusion coefficients for auxin a or b. The evidence suggests that this discrepancy is due not to an auxin of different molecular weight, but rather to the presence in the extracts of substances which partially mask the biological effect of the auxin on Avena coleoptiles. At least some of these substances are ether and water soluble, with molecular weights larger than auxin. Partial purification of auxin extracts can be made by diffusion methods. When determining the activity of unknown extracts, the possible presence of such masking substances should not be overlooked. If they are present in appreciable amounts, the actual quantity of auxin in an extract may be greater than that detected by the Avena test.

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## THE CAUSE OF NATURAL PARTHENOCARPY 1

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THAT IT is possible to produce parthenocarpic fruits artificially by treating the pistil of a flower in the bud stage with growth promoting substances or auxins, as they will be called in this paper (Went and Thimann, 1937), has now been shown by several investigators (Gustafson, 1936; Hagemann, 1937; Gardner and Marth, 1937). From this fact and the fact that auxin is known to be widely distributed in the higher plants we may assume that auxin naturally plays a part in the growth of a fruit. If so, where does it come from?

Suggestions have been made that the auxin comes from the pollen or the developing seeds (Dollfus, 1936; Gustafson, 1937, 1938a). Laibach (1932) and Thimann (1934) have shown that pollen contains auxin. As to the latter suggestion, it will suffice to point out that there are fruits without seeds such as the orange, lemon, grapefruit, banana, grape, cucumber, and others. Recently Gustafson (1938a, 1938b) proposed the hypothesis that the reason some plants produce fruits parthenocarpically is that the ovaries of these plants contain, in the flower bud stage, enough auxin to cause them to commence growing without fertilization, while the ovaries of other plants commence growing only after pollination and fertilization have augmented the auxin supply already in them. We may then think of the usual fruit growth as being initiated by pollination and fertilization, which brings into the ovary a sufficient quantity of auxin from the pollen grains and pollen tubes to start the enlargement of the ovary (Gustafson, 1937), and continued by the additional auxin produced by the developing embryos and seeds, which diffuses into the ovary

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to supply what is needed. Contrasted with this is the formation of a fruit from an ovary which contains enough initial auxin within itself to start enlargement without any additional being derived from pollination and fertilization, the resulting young fruit either producing its own auxin or obtaining it from the leaves. The fruits produced in this way are parthenocarpic.

The purpose of this investigation was to compare the auxin concentration in the ovaries, at flower bud stage, from plants that produce fruits parthenocarpically and from plants that require fertilization.

PROCEDURE.—The work here reported was done at the William G. Kerckhoff Laboratories at the California Institute of Technology. The citrus material was obtained either from the Citrus Experiment Station at Riverside or from the Armstrong Nurseries at Ontario, California. All the grape material came from the Armstrong Nurseries. The growth hormone content was determined by the Avena method, the technique of which has been discussed in detail by Went and Thimann (1937).

The auxin was extracted with freshly distilled ether according to Van Overbeek's (1938) modification of other methods. The dried residue was thoroughly mixed with a small amount (usually 0.4 to 0.8 cc.) of 1.5 per cent agar, and after standing 90 minutes was poured into a nickel plated brass mold  $1.5 \times 8.0 \times 10.5$  mm. in size. After the agar had been cut level with the surface of the mold, the large block was cut into 12 equal pieces. The material was now ready for the test. Great care was taken to have freshly prepared extracts. Usually the extract was prepared the same day that it was used, but if it could not be used the same day that it was made, it was always stored in a refrigerator in dried condition. The extraction was always carried out in the dark and usually, though not always, at a low temperature.

Control experiments in which Avena plants were treated with a known concentration of indole acetic acid (usually 21.5 gammas per liter) were run with every experiment. By this means the sensitivity of the test plants was determined for each experiment, which was very important because there was considerable variation day by day, and the auxin

Table 1. Auxin content in the ovaries of unopened flowers and in young fruits of oranges and lemons.—The auxin concentration is denoted in terms of indole acetic acid equivalents, and the figures are gammas per kilogram of fresh material.

	The navel		Valencia						
			Paper rind		Seedless		Satsuma	Eureka lemon	
	Washington (seedless)	Robertson (seedless)	(Seeded)	Seeded	Armstrong	Rico No. 1	Seedless	Seeded	Seedless
Buds Fruits:	0.73	1.16	0.58	0.58	2.39	1.16	4.01	0.43	0.78
2 wee		1.06 0.61	1.35 0.81	2.55 0.74	0.99	•••	1.71	3.00	•••

concentration in a plant can be calculated in terms of indole acetic acid (see Van Overbeek) rather than in terms of some of the many units which have been employed by different investigators.

A few experiments were performed in which the plant material was placed on the agar blocks as in Went's original experiments. In most instances such blocks when placed on Avena coleoptiles caused no curvatures. It had been hoped that this method could be used, but since so few plants gave any results it was abandoned for the extraction method.

RESULTS AND DISCUSSION.—It very soon became obvious that one could not profitably compare unrelated species with one another because the difference in the auxin content in the vegetative parts of different species varies greatly. Therefore it was necessary to obtain varieties of the same species that were parthenocarpic and non-parthenocarpic. Fortunately, the nearby Citrus Experiment Station at Riverside had a great variety of oranges, some producing seeds and others not. Through the courtesy of Dr. H. B. Frost an abundance of flower buds and young fruits were obtained from several varieties that were selected after a careful investigation of relationships. Two

varieties of oranges were also obtained from the Armstrong Nurseries at Ontario through the courtesy of Dr. W. E. Lammert, and the Seedless Valencia Rico No. 1 was obtained from Mr. C. S. Pomeroy of the U. S. Department of Agriculture at Riverside.

The varieties finally used were the seedless Navel varieties Washington and Robertson, the latter being a sport of the former; Paper rind, a seeded form; three varieties of Valencia—the ordinary seeded variety, a seedless sport of the seeded variety called Armstrong, and a seedless form called Rico No. 1, which was introduced from Porto Rico and may not be related to the Valencia, though it looks like it. These six varieties are very closely related to one another. Since all belong to Citrus sinensis, they can properly be compared. Satsuma, a seedless variety of C. nobilis, was also used, but it cannot be compared directly with the others. After it was discovered that there existed a tree of seedless lemons (Citrus Limonia) in Riverside, Eureka lemon was also used.

It must be stated that authorities on the subject are not agreed as to whether pollination is or is not necessary for the fruit production in oranges. Evidence points towards the lack of necessity for

Table 2. The amount of fresh material used of each variety, the quantity of 1.5 per cent agar with which the dry residue was mixed, the mean curvature of 24 Avena coleoptiles treated with this agar, the mean curvature of 24 control Avena coleoptiles treated with indole acetic acid (21.5 gammas per kg.), and the concentration of auxin in terms of indole acetic acid equivalents in gammas per kilogram fresh material as calculated by the formula  $c \times s \times v/w$ , (Van Overbeek, 1938), where c is the curvature of the experimental plants, s the sensitivity of the Avena plants or the number of gammas of indole acetic acid required to produce one degree of curvature, v the volume of agar with which the residue was mixed, and w the fresh weight of the material used.

	Orang	es				
	Eureka	lemon	Washington navel	Paper rind	Valencia	Indole acetic acid
Notation	Seeded	Seedless	(seedless)	(seeded)	(seeded)	$(21.5\gamma)$
Weight in gm	18.80	18.45	32.10	19.21	18.15	
Agar cc	0.6	0.6	1.0	0.6	0.6	
Curvature in degrees	7.8	9.0	9.0	7.8	8.3	9.1
Sensitivity						2.36
Concentration of auxin						
per Kg	0.57	0.67	0.65	0.56	0.63	

pollination, but it is not conclusive. For this reason care was taken to use only unopened flower buds, so that there could be no possible question about the influence of pollination upon auxin content of the ovaries.

Usually about 200 large buds which were nearly ready to open were collected from each variety during the middle of the day. Within a few hours the ovaries had been removed from the buds, weighed, and placed in freshly distilled ether. The extraction time was usually two days, during which time the material was kept in an electric refrigerator. Table 1 is a composite of three such experiments.

Table 1 is a composite of several experiments, and it would be very difficult to include in it certain information which the reader might wish to know. Therefore the first experiment with lemons and oranges is given in detail in table 2. It will be noticed that not all of the oranges were used in this experiment, but nevertheless has been chosen because it is the first one and is typical of the others.

In this experiment the differences between the seeded and the seedless varieties were not nearly so large as they were in the second experiment where the figures were as follows: seedless lemon 0.70, seeded lemon 0.12, Washington navel 1.05, Valencia 0.51, Rico No. 1 (seedless) 1.16, Paper rind (seeded) 0.45, and Satsuma (seedless) 4.01 gammas per kilogram fresh material.

Table 1 shows that in every instance the auxin content of the ovaries of the buds is higher in the parthenocarpic variety than in the corresponding seeded variety. On the other hand, after the ovaries have started to grow and the fruits are two or four weeks old, the auxin concentration has increased in the seeded varieties, while in the seedless varieties, where measured, it has decreased. Besides bringing out the fact that the auxin content is higher in the ovaries of the parthenocarpic varieties than in the non-parthenocarpic, the table also shows that fertilization and seed development increase the auxin concentration of the ovary. This increase is in agreement with the suggestion previously made that the seeds supply the auxin needed in the growth of the ovary into a fruit.

It should further be noticed that the Robertson Navel has a much higher auxin concentration than the Washington. This may be associated with the greater early growth rate of the former. When four weeks old the Robertson fruits are several times as large as the Washington, and they also mature considerably earlier. Both the seedless varieties of the Valencia have a much higher auxin content than the common seeded variety, and the Armstrong, which is known definitely to be a bud sport of the seeded Valencia, has an auxin concentration four times as large as that of its parent type. The Satsuma has a surprisingly large auxin content, but unfortunately there is nothing to compare it with except in a general way.

Diffusion experiments were also tried, but only the lemon gave any results. One millimeter thick 2 per cent agar sections were cut into blocks 8.0 X 10.5 mm., and these were subdivided into 12 equal pieces which were separated from one another on a moist slide. On top of each small block was placed an ovary, and the slide was then left in a damp Petri dish for two hours. The ovaries were removed from the agar blocks, which were placed on Avena coleoptiles in the usual way. It was found that the average curvature of 24 Avena coleoptiles treated with agar blocks upon which had rested the ovaries from the seedless varieties was  $6.3 \pm 0.7^{\circ}$ , while the average curvature of 24 other Avena coleoptiles treated with agar blocks upon which had rested ovaries from the seeded variety was only  $3.3 \pm 0.6^{\circ}$ . The actual concentration was not obtained in this experiment, but Went has found that the curvature of the Avena coleoptile is proportional to the auxin concentration in the agar block with which it was treated. Therefore there is a much greater auxin concentration in the ovaries of the seedless variety than in those of the seeded variety. This is in agreement with the data from the extraction experiment with lemons.

Three varieties of grapes, Thompson and Black Manukka seedless and seeded Muskat, were obtained from the Armstrong Nurseries. The exact relationship between these three varieties is not known, but it is thought by authorities to be fairly close.

Material was collected in the flower bud stage, and the whole flower buds were used in extraction. The procedure of extraction and testing was the same as that for the oranges. Table 3 gives the results for the one test that was made with flower buds.

Table 3. Auxin content in flower buds of three varieties of grapes. Auxin concentration is denoted in terms of indole acetic acid equivalents and the figures indicate gammas per kilogram of fresh material.

	Seedless	Seeded
Thompson	Black Manukka	Muskat
2.74	1.30	0.34

The auxin content is much higher in the seedless varieties than in the seeded variety.

The three experiments with oranges extending over the whole blooming period, three experiments with lemons, one of which was a diffusion experiment, and one experiment with grapes all bring out the same fact—namely, that the ovaries of the varieties that are parthenocarpic have a much higher auxin concentration in the flower bud stage than those that develop into seeded fruits.

Conclusion.—While it is true that only three species of plants that are naturally parthenocarpic

have been investigated, yet the auxin content in the ovaries of all the parthenocarpic varieties is so much greater than in the non-parthenocarpic that there seems to be no question about this being a general condition. We can, therefore, at least tentatively or until contrary information is obtained in other species, accept the hypothesis that the reason some fruits develop without seeds is that they have a high auxin content in the ovaries at the time of blossoming and that this is high enough to set off the growth processes with the result that the ovary commences to grow even though there has been no fertilization. After growth has once commenced, it is continued either because auxin is produced in

the ovary itself or because auxin is transported into it from the leaves.

#### SUMMARY

It has been shown that the auxin content in the ovaries of flower buds from varieties of oranges, lemons, and grapes that produce fruits parthenocarpically is higher than in the ovaries from corresponding varieties that do not produce fruits parthenocarpically.

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# A NEW TYPE OF EMBRYOGENY IN THE CONIFERS 1

# Phyllis L. Cook

HAVING RECENTLY completed a study of the early embryogeny of Juniperus communis, I believe that the results are worthy of publication in that they show a type of embryogeny hitherto unknown in the Conifers. Ottley (1909) studied the life history of J. communis through the first division of the zygote, and Noren (1907) and Nichols (1910) studied it through the development of the proembryo. Strasburger (1872) figured one embryo from J. communis as it appears during the first year and a single embryo from a mature seed from the same species. He also figured the proembryo and the developing multicellular embryo of J. virginiana. The process of development from the proembryo stage to the beginning of the multicellular stage is often long and complicated, and with the one exception of Strasburger's figure, no description of the process in any species of Juniperus has existed in the literature until the present. It is between the proembryo and the multicellular stages that the unique features occur.

During the last two summers, I have collected J. communis in the Muskoka Region of Ontario, where it is native, and on the campus of the University of Illinois, where it is cultivated. I have

<sup>1</sup> Received for publication December 27, 1938. To Dr. J. T. Buchholz I am indebted for direction and criticism of this study. had the privilege of studying mounted embryos of *J. communis* collected at Cold Spring Harbor, Long Island, by Dr. J. T. Buchholz, and of *J. mexicana* from Austin, Texas, also collected by Dr. Buchholz. For the study of fertilization, the proembryo, and the late embryo stages, I have used microtome sections, and for the rest of the embryogeny I have employed the Buchholz (1938) method of mounting whole conifer embryo complexes.

INVESTIGATIONS.—In Illinois fertilization takes place during the first or the second week in June, and in Ontario during the last week in June. One, two, three, or occasionally more eggs in each archegonial complex are fertilized. Nichols (1910) states that normally eight free nuclei are present before the proembryo becomes cellular, and my findings agree with his. The complete proembryo consists of about twelve cells arranged in three more or less definite tiers. The nuclei of the upper tier are never completely walled in, and they take no part in further development; the cells of the middle tier elongate, pushing the cells of the lowest tier out of the archegonium into the nutritive tissue of the gametophyte. In the cavity which they form in the gametophyte, the cells which constituted the two lower tiers of the proembryo begin a process of development which is unique in the Coniferales and

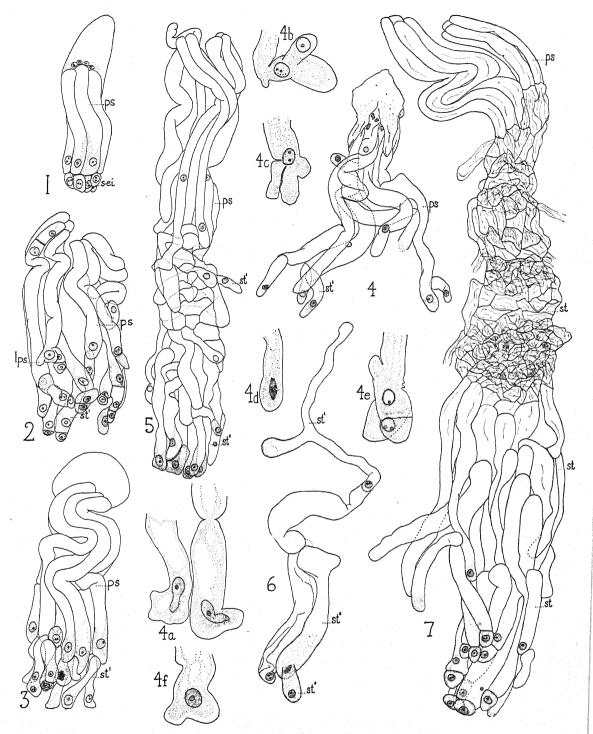


Fig. 1-7. Juniperus communis; drawn from dissected and mounted material with the aid of a camera lucida.—Fig. 1. Embryo complex just emerging from the archegonial complex. ps., prosuspensor; s-ei, suspensor-embryo initial. ×115.—Fig. 2. Young embryo complex showing the embryonic nature of the prosuspensor cells. lps, lobed prosuspensor; st¹, suspensor-tube¹. ×115.—Fig. 3. Young complex showing the more usual condition at this stage, prosuspensors equally elongated, lower tier of cells almost equally elongated also. ×115.—Fig. 4. Young complex in which the prosuspensor cells have pushed aside the cells of the lower tier and are behaving as embryo initials. ×115.—Fig. 4a, b, c, d, e, and f. More highly magnified ends of elongated tubes showing variety of lobing and nuclear conditions. ×252½.—Fig. 5. Complex one month after fertilization, showing prosuspensor and two tiers of elongated tubes. Drawn in only one plane of focus. ×115.—Fig. 6. The product of one terminal embryo unit, showing lobing of three tiers of tubes. ×115.—Fig. 7. Complex five months after fertilization, number of tiers of tubes indefinite, approximately four or five, end-cells probably about to become multicellular. ×115.

approximated elsewhere only in the Gnetales (Land, 1907; Thompson, 1916; Haining, 1920; fig. 13). It immediately becomes apparent that each cell of the elongated tier as well as each cell of the lowest tier is a potential embryo initial, for both tiers elon-

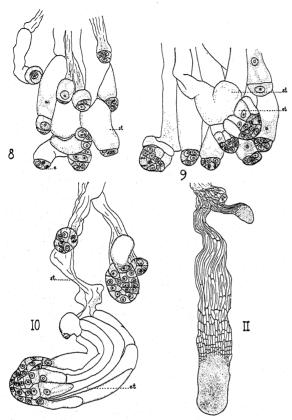


Fig. 8-11.—Juniperus communis.—Fig. 8. Multicellular embryos forming on the ends of the sixth tier of tubes which are swelling considerably. st, suspensor tube; e, embryo. Seven months after fertilization. ×230.—Fig. 9. Multicellular embryos eight months after fertilization; embryonic cells elongating backwards; et, embryonal tube. ×230.—Fig. 10. The dominant embryo determined seven months after fertilization. ×230.—Fig. 11. Massive embryo with an aborting embryo left behind. Seven months after fertilization. ×54½.

gate similarly. In this elongation the cells of the middle tier sometimes push aside those of the lowest tier because of a difference in their rates of elongation (fig. 4). It is more usual, however, for the lowest tier to maintain its position in spite of the more rapid elongation of the tier above, and consequently its cells are pushed ahead (fig. 3). It should be remembered that the products of each zygote are undergoing the same process; so that the number of embryonic units present in an ovule at this stage is theoretically eight times the number of the original zygotes in the complex. All the cells continue to elongate, but because of their more favorable position at the start, the cells of the lowest tier are likely

to reach farthest into the nutritive tissue of the gametophyte. A number of them are usually tied for first place; so that at the very beginning of sporophytic life, there is keen competition among individuals (fig. 3). Occasionally when individual units elongate at varying rates, the resulting complex may be more U- or V-shaped (fig. 2) than truncate in outline (fig. 3); and then the units farthest advanced may immediately give rise to multicellular embryos. The truncate type of complex, however, is more common; and when this is the condition, the lowest cells instead of dividing and giving rise to multicellular embryos, elongate and often lobe (fig. 4a, b, c, d, e). Two or even three lobes are frequent, each projecting in a different The single large nucleus of a tube may migrate into one of the lobes, or it may divide by mitosis and provide two or more lobes with nuclei (fig. 6). Lobes and unlobed ends of tubes then intertwine, forming a compact knot. This knot of tubes bores its way into the gametophyte tissue by means of two processes—the chemical process of digestion and the mechanical process of elongation. The rate of elongation exceeds that of digestion, so that the ends of the tubes are thrust into undigested. compact tissue. This condition makes it difficult to dissect out the complex of tangled tubes cleanly, and after dissection the finger-like ends of the tubes usually still hold fast to masses of nutritive cells. Sometimes nucleated lobes segment from the rest of the tube (fig. 6), but often they remain open. After the tubes grow to a certain length (15 to 20 microns), the nucleus divides, and a wall between the two daughter nuclei forms a rounded cell on the end of the tube, which may now perhaps be called a suspensor. If there is not too keen competition at this point, the rounded cells may give rise to multicellular embryos, but they in turn more frequently elongate into a second set of suspensor-like tubes without end-cells. If the rate of their elongation is unequal, they will cut off cells at their tips, the farthest advanced of which will divide and form a mass of cells; but, if as is again usual, there is still a tie among a number of tubes, they again lobe and intertwine before cutting off new cells (fig. 6). Some tubes are left behind during each of these advances because of their slow growth (fig. 5, 7), but since the tubes that compete successfully during the early laps of the race are likely to lobe, the number of tubes in competition may not be appreciably diminished for some time. The lobing process is apparently more or less confined to the early sets of tubes, but the elongating-intertwining process may be repeated an indefinite number of times, forming a fairly recognizable tier of tubes for every occurrence (fig. 7).

As each new set of suspensor-tubes develops, those preceding it begin to collapse; so that the entire elongated complex resembles a much twisted and tangled rope of spaghetti (fig. 7). The elongated cells that constituted the middle tier in the proembryo and the next to the last formed tier of tubes remain fairly turgid, while the very last tubes are extremely round and plump (fig. 7, 8). The nuclei present in the tubes after the end-cells are cut off are large at first, but they soon disintegrate (fig. 7).

Eventually because of varying rates of elongation and the loss of numerous tubes, the competition among potential embryos does become less keen than at first, and the tubes that have penetrated the farthest cut off end-cells that give rise to multicellular embryos (fig. 8). Mitoses take place, and the apical cell method of growth is established (fig. 8). The single suspensor-tubes swell to an enormous size at this stage (fig. 9). The gametophyte tissue is continuously being digested away; but the embryos proper are nearly always located a little ahead of where the digestion has been the most complete, which would suggest that it is the tubular suspensor cells that secrete the digestive enzymes perhaps more than the cells of the embryo itself; or the condition may only be due to the fact that digestion is slower than elongation. When the embryo consists of about a dozen cells, those cells on the outside and farthest from the growingpoint elongate backwards, forming a massive secondary suspensor and also pushing back any embryos that may still be competing (fig. 10). Since the number of tiers of suspensor tubes is indefinite, the time of multicellular embryo formation also varies; so that in a batch of ovules collected from the same plant six months after fertilization, it is possible to find embryo complexes with embryos in all but the last stages of differentiation. Thus there is no definite wintering-over condition as Strasburger (1872) supposed there was.

Although several embryos in an ovule may become multicellular, more than one that is completely differentiated is rare in a mature seed. Aborted embryos are often found entangled with the collapsing suspensor of the successful one (fig. 11). The mature embryo normally has two cotyledons, and in the two growing seasons required for its full development, it replaces most of the gametophyte tissue with its own bulk.

Discussion.—This investigation of Juniperus communis has disclosed a type of embryogeny different from that found in any of the other genera of the Coniferales. In no other genus of the order do we find the lowest cells of a proembryo elongating without first cutting off embryonic cells at their tips. As long ago as 1872, Strasburger described the tangled skein of tubes in the ovule of Juniperus communis and remarked that in dissecting the complex, it was easy to lose the end-cells. He probably saw the elongating suspensor-tubes in many of his dissections, and thought that he had lost off the terminal cells. This is perhaps true in complexes dissected some weeks after fertilization; but in the early stages I believe that nothing is lost, because

when there is close competition among embryonic units, they elongate before cutting off end-cells, so that there are no small cells present to be lost.

In the organization of the first products of the zygote and in their subsequent behavior, J. communis is similar to Ephedra trifurca as described by Land (1907) and to Gnetum as described by Thompson (1916) and by Haining (1920). In

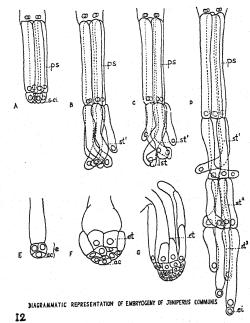


Fig. 12. Juniperus communis. Diagrammatic representation of the embryogeny of the product of one zygote. A, proembryo of three tiers. B, prosuspensor cells and terminal cells elongated. C, terminal cells, elongating, lobing, intertwining, two tubes being left behind. D, prosuspensor tier and three tiers of elongated tubes, competition reduced in the last tier, terminal cells cut off and about to produce multicellular embryos. E, most advanced cell of D becoming multicellular, apical cell prominent. F, further development of E, suspensor tube swollen and embryonal tubes forming secondary suspensor. G, further development of F, massive embryo. ps, prosuspensor; s-ei, suspensor-embryo initial; st<sup>1, 2, 3</sup>, suspensor tube <sup>1, 2, and 3</sup>; lst<sup>1</sup>, lobed suspensor tube; ei, embryo initial; ac, apical cell; e, embryo.

E. trifurca the fusion nucleus gives rise to eight free nuclei. Three to five of these with their surrounding cytoplasm become separated from the rest of the cytoplasm by walls, and each one is a potential embryo initial. The nucleus divides, forming two nuclei which become unequal in size. A large vacuole forms in the upper part of the cell, and two lobes are put out by the cell wall at points nearest the nuclei. The lobe near the larger nucleus elongates, and the other disappears. After the tube has elongated, the larger nucleus moves down into it almost to its tip, and a cleavage ring forms behind and separates the cytoplasm into two masses. The smaller nucleus then migrates down the suspensor-tube (fig. 13, A, B, C, D, E).

In Gnetum sp. 33 (Thompson, 1916), the fusion nucleus gives rise to a group of cells, each of which is a potential embryo initial. Each initial elongates apparently without the division of the nucleus and penetrates deeply into the gametophyte. Although Thompson has sometimes seen two or more nuclei in a tube, he believes that "a single enormously large nucleus" is usually the only one present. In this species he never saw walls and very little branching of the tubes. In G. gnemon only two cells are formed by the division of the fusion nucleus, and each one is an embryo initial.

In G. funiculare Haining (1920) did not see early post-fertilization stages, but her figures F, G, and H

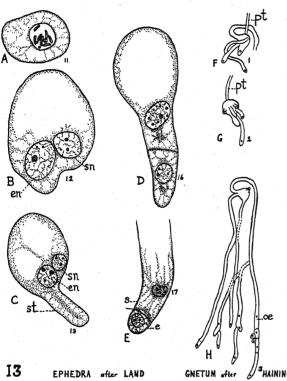


Fig. 13. Land's (1907) figures 11, 12, 13, 16, 17 of Ephedra trifurca, showing the early development of one embryo initial. Note the elongation of the tube and the migration of the nucleus. ×250.—Haining's (1920) figures 1, 2, 3 showing the branching and elongation of tubes in one embryo initial in Gnetum funiculare. Note the absence of small terminal cells. ×25.

of my figure 13 show "a large, single-celled proembryo" from which several "suspensors" have grown. The nucleus has divided, and one daughter nucleus has migrated to the tip of each tube. Each tube branches, and cross-walls separate the branches from the main tube and also any two nuclei in one tube.

In Juniperus communis the fusion nucleus gives rise to about twelve nuclei, eight of which become completely walled in. All eight are potential embryo initials. The four of the upper tier assume the function of suspensors and push those of the

lower tier out of the complex. The nuclei of the embryo initials that become suspensors do not divide, but the cells elongate and sometimes lobe. It is common for the embryo initials of the lower tier to elongate and lobe, and the nucleus of each may migrate into one tube, or it may divide and supply two or more tubes.

In the comparison of these four species, it becomes apparent that there is confusion in the use of the term "proembryo." In describing the embryogeny of the Coniferales, Chamberlain (1935) uses the term "proembryo" as a collective name for the products of a single zygote before they break through the base of the egg. In describing Ephedra, Land (1907) uses the same term to designate each of the eight cells formed by the division of the zygote, and Thompson (1916) describing Gnetum, uses the term to cover the whole group of cells formed by the division of the zygote. It is clear from this study that the constituent cells of the "proembryo" of Chamberlain and Thompson are the homologues of the "proembryos" of Land and that the eight walled cells of Juniperus are the homologues of the three to five cells of Ephedra, of the indefinite number of walled cells in Gnetum sp. 33, and of the two cells in G. gnemon. If we speak of all these homologous units as embryo initials, we may avoid some confusion. The subsequent elongation of the individual embryo initials is similar in Ephedra trifurca, Gnetum sp. 33, G. gnemon, and in Juniperus communis, except that in Ephedra the nucleus divides prior to the elongation of the cell. Although Juniperus is unique in the Coniferales in the behavior of the lowest embryo initials, many features of its embryogeny are found elsewhere in the Cupressaceae. The products of the fusion nucleus before they break out of the egg are arranged similarly in Juniperus, Thuja, Biota, Cupressus, Libocedrus, and Chamaecyparis. Like all these genera except Thuja, Juniperus exhibits cleavage polyembryony, but the cleavage takes place very early. Often the cells of the middle tier separate as they do in Chamaecyparis (Buchholz, 1932), so that embryos sometimes form on the ends of cells of both tiers.

The affinities of the Gnetales have always been in question. Land could see no proof for their relationship to any living group, but he recognized that they had many characteristics of the Coniferales, and he thought they had more in common with the Taxaceae than with any other family. He did not state specifically what characteristics he was considering. Arber and Parkin (1908) believed that the Gnetales had been derived from Cycadalean stock on a basis of their floral organization. Thompson's (1912) studies have led him to favor Coniferous relationship, although he has not seen proof for a Gnetalean descent from any modern group of Conifers. Among the points of similarity between the two groups, he mentions the arrangement and structure of the tracheid pits, bars of Sanio, tertiary spirals, trabeculae, primitive uniseriate and lignified rays, lack of centripetal and presence of centrifugal wood in the leaf-trace, and the structure of the vascular bundles of the leaves. Buchholz (1920) called attention to the occurrence of cleavage polyembryony in the Gnetales and suggested that this feature connects the Gnetales with the conifers rather than with the cycads. Again Buchholz (1929) referred to the similarity between Coniferous and Gnetalean embryogeny and remarked that "the character of the proembryo (of Ephedra) fits in very beautifully as a derivative of some form having an early embryogeny resembling that of Biota." His hypothetical form might well be Juniperus, for the present study of J. communis makes possible an easy transition from the typical Cupressacean embryogeny to that of the Gnetales; so that the organization of the products of the fusion nucleus and the elongation and branching of the embryo initials may now be added to Thompson's list of similarities between the Coniferales and the Gnetales. In the 1929 paper, Buchholz also mentioned the opposite or whorled arrangement of the leaves in the Cupressineae and the Gnetales. I have found no mention in the literature of two other similarities between the groups—namely, (1) the fact that the cells lining the micropylar cavity are large and elongated at right angles to the axis in Gnetum and in Juniperus and (2) that pollen grains germinate in the micropylar cavity in Gnetum and sometimes, at least, in Juniperus. It seems, therefore, that the Gnetales are related to the Coniferales and that the relationship exists between

them and one of the living Coniferous families, the Cupressaceae.

#### SUMMARY

The early embryogeny of Juniperus communis is described, disclosing a type of embryogeny unique in the Coniferales and approximated elsewhere only in the Gnetales.

The products of division of the fusion nucleus organize in three tiers of four nuclei each, the lower eight of which are completely walled in. The eight walled cells are embryo initials which elongate without first cutting off end-cells. The elongated cells lobe, intertwine, and then cut off end-cells. elongating-intertwining process may be repeated an indefinite number of times, depending upon the keenness of competition among individual embryonic tubes. When competition almost ceases, the tubes farthest advanced cut off end-cells that give rise to multicellular embryos, which show apical cells.

In a batch of ovules collected from the same plant six months after fertilization, it is possible to find embryos in all but the last stages of differentiation.

The embryogeny of Juniperus communis is similar to that of Ephedra and Gnetum in (a) the organization of the products of the division of the fusion nucleus, (b) the early establishment of cleavage polyembryony, and (c) the elongation and branching of the embryo initials. The similarity of embryogeny may be taken as additional evidence in favor of a relationship between the Coniferales and the Gnetales through the Cupressaceae.

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# EXPERIMENTAL METHODS FOR THE STUDY OF THE ROLE OF COPPER, MANGANESE, AND ZINC IN THE NUTRITION OF HIGHER PLANTS <sup>1</sup>

P. R. Stout and D. I. Arnon

In investigating the rôle of zinc, manganese, and copper in plant nutrition, the use of suitable experimental methods for growing plants assumes particular importance. The amounts of these so-called microelements required by plants are so small that they are frequently supplied in considerable part or in toto by the incidental impurities included in the culture medium. A successful demonstration of the indispensability, as well as any study of the quantitative aspects of the importance of microelements in the nutrition of higher plants, depends directly on the removal of contaminants from the culture medium.

The purpose of this investigation was to develop an experimental technique which, with the use of ordinary laboratory and greenhouse facilities, would permit the undertaking, on a fairly large scale, of experiments to study the physiological importance of zinc, manganese, and copper in the nutrition of higher plants. The object was attained by using the water culture method and, by proper selection of materials and purification procedures, reducing the contaminations derived from water, chemicals, and containers. The use of very sensitive chemical tests to determine the amounts of contaminants present in the culture medium after purification made it possible to prepare nutrient solutions of equal degrees of purity. It was possible, using this technique, to obtain consistent and reproducible responses in plants from adding minute amounts of metals to the nutrient medium (for example, 1 part of zinc in 200,000,000 parts of culture solution, which amounted to  $1\gamma$  [0.001 mg.] of zinc to a plant).

GENERAL CONSIDERATIONS.—The metal contaminants found in nutrient solutions are derived from three sources: containers, water, and chemicals. Previous investigators (Steinberg, 1919, 1935; Hopkins, 1933), studying the effect of microelements on lower plants, have described successful methods for nutrient solution purification in which metal impurities were removed by precipitation and adsorption at high temperatures and slightly alkaline reactions in the presence of calcium and phosphate ions. They have applied these purification procedures to either the complete nutrient solution (Steinberg, 1919, 1935) or to one divided into two portions (Hopkins, 1933), removing in one or two operations such impurities as were derived from the several nutrient compounds, water, and con-

<sup>1</sup> Received for publication January 9, 1939.

In this investigation in which large scale experiments with higher plants were undertaken, the purification of the complete nutrient solution was found to be impracticable because of the relatively large volumes required (usually several hundred liters of nutrient solution in one experiment). Furthermore, as a result of transpiration, water, in large amounts, had to be added to the cultures before the nutrient salts needed replenishing. This was a significant consideration, since, as will be shown later, ordinary distilled water was found to be a source of metal contamination.

It was attempted, therefore, to remove metal contaminants in the nutrient medium at their source by selecting suitable containers and purifying water and each nutrient salt separately. Individual purification procedures, based on Steinberg's technique (Steinberg, 1919, 1935), were adapted for each molar stock solution of nutrient salts. In addition, large quantities of distilled water were purified by redistillation, using a Pyrex glass condenser, and Pyrex glass containers were adopted for growing plants.

After purification the purity of water and the various molar stock solutions of nutrient salts was determined by means of an adaptation of the diphenylthiocarbazone (dithizone) test. Dithizone forms colored complexes at alkaline reactions with a number of metals (Snell and Snell, 1936) and provides a very sensitive test for zinc, copper, nickel, cobalt, lead, mercury, cadmium, thallium, and bismuth. This test, as described later, permitted the detection of 0.0005 mg. and, with special care, 0.0001 mg. (0.1 of a gamma) of the combined metals listed above.

The purification procedure reduced the metal content of the nutrient salts from 500-5,000 to consistently less than 10 parts per billion, and the redistillation reduced the metal content of distilled water from 10-100 to consistently less than 1 part per billion. Each lot of water and stock solution of chemicals was tested and, if found pure, approved for future use in the preparation or the replenishing of nutrient solutions. The purified chemicals and water could thus be added independently of each other, to give nutrient solutions of equal and reproducible purity.

This investigation was directly concerned with three elements: zinc, copper, and manganese. The dithizone test, as used here, while measuring the combined amounts of zinc and copper along with the other metals listed above does not include manganese. It was found, however, by the persulfate test for the determination of manganese, that this metal was also removed in the purification process

<sup>&</sup>lt;sup>2</sup> Steinberg (1919, 1935) and Hopkins (1933) used Pyrex glassware as containers, which, as will be shown later, do not serve as a source of contamination.

along with copper and zinc. The dithizone test was adopted, therefore, as a general test for determining the removal of the aggregate metal impurities, including manganese, from each lot of purified chemicals and redistilled water.

Selection of containers for growing plants.—In preliminary experiments, various containers of black iron, porcelain, and stoneware were tested for freedom from metal contamination. These containers were coated with asphalt paint, which was found to be free from metal impurities, and were allowed to stand filled with pure water for a five-week period. From 20 to 200 gammas of metallic impurities (dithizone test) were brought into solution in this period of time, more than enough to supply the needs of plants in some cases.

No paint was effective in preventing metal containers from yielding impurities to the solution. Most paints, the asphalt (black asphaltum) paint being an exception, are themselves a ready source

of contamination.

It was known from previous experience that Pyrex glassware, as distinguished from Fry, Jena, or soft glass was satisfactory for storing various metal-free reagents. Pyrex containers have previously been used with good results in other investigations on microelements (Sommer and Lipman, 1926; Chapman et al., 1937), and, since they were not a source of metal contaminants, they were adopted in all further work. Two and four liter beakers were used. The covers, cast from plaster of Paris and soaked in hot paraffin, contained several holes in which the plants were supported with The beakers were covered with thick paper to exclude light. Recently shallow rectangular Pyrex ovenware dishes of approximately two liter capacity, with glass covers, were adopted for some experiments. The plants were supported with cotton in holes drilled in the glass covers. Both the glass dish and the cover were painted on the outside with black asphalt followed by aluminum

PREPARATION OF WATER FREE FROM METAL IM-PURITIES.—Repeated tests have shown that ordinary distilled water, from various sources, contained metal contaminants varying from 0.1 to 0.01 part per million. These impurities were traced to the generally used tin-lined copper stills and metal piping through which the distilled water circulated and also in some cases to an unsatisfactory design and too rapid rate of operation of the still, resulting in carrying over unvaporized water particles.

No consistency was found in the amounts of impurities present in different samples of water derived from a given source. Not only did water secured from different stills, or from different taps on a line from one still, contain variable amounts of metal contaminants, but also different samples drawn from the same tap in the course of a day varied in their metal content from 100 to less than 1 part per billion.

Redistilled water free from metal impurities was obtained by using a Pyrex glass trap and a Pyrex condenser and operating the still at a rate of distillation sufficiently slow to give a cool distillate.

The pure redistilled water was stored in Pyrex carboys. Each lot was tested by the dithizone test as described below.

Purification of nutrient salts.—There was no consistent relation between the market grade of salts and their freedom from metal impurities. C. P. salts from various sources contained variable amounts of metal impurities, which in certain cases exceeded those present in salts of technical or even fertilizer grade. For example, a reagent grade of potassium nitrate was found to contain 100 ppm. of metal impurities, whereas a fertilizer grade of the same salt contained 0.1 ppm. With another batch of salts entirely different amounts of impurities were found. Since all the salts were purified before use, the cheapest grade was selected in each case, provided it was free from otherwise objectionable impurities (phosphate fertilizers, for example, frequently contain appreciable amounts of fluorides).

Table 1. Nutrient salts used for purification.

Salt	Grade	Remarks
Calcium nitrate Ca(NO <sub>3</sub> ) <sub>2</sub> ·2H <sub>2</sub> O <sup>a</sup>	Fertilizer	Singularly free from metal impurities
Potassium nitrate KNO <sub>3</sub>	U. S. P.	Singularly free from metal impurities
Magnesium sulphate MgSO <sub>4</sub> ·7H <sub>2</sub> O	U. S. P.	99.9% pure
Di-Ammonium phosphate (NH <sub>4</sub> ) <sub>2</sub> HPO <sub>4</sub>	C. P.	Technical grades free from fluorides are also satisfactory
Di-Potassium phosphate $K_2HPO_4$	C. P.	Technical grades free from fluorides are also satisfactory
Ammonium sulphate (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	C. P.	

a Found by analysis to contain this proportion of water.

The kind and grade of salts used in this investigation are given in table 1. Molar stock solutions of the salts were prepared for making the nutrient solution employed in this laboratory (Hoagland and Snyder, 1933; Arnon, 1938) as well as for certain special nutrient solutions involving ammonium sulphate. As the monobasic potassium and ammonium phosphate included in these nutrient solutions cannot be treated with calcium carbonate in the purification process, the di-basic forms— $K_2HPO_4$  and  $(NH_4)_2HPO_4$ —were used. The latter were acidified after purifications with pure C. P.  $H_2SO_4$  or  $HNO_3$  (see directions below) before being added to the nutrient solution.

A supplementary iron solution containing 0.5 per cent FeSO<sub>4</sub> and 0.5 per cent tartaric acid was

added twice weekly at the rate of 0.5 cc. per liter of nutrient solution. The nutrient solutions were supplemented with the A4 and B7 solutions of microelements (Arnon, 1938), for the preparation of which tested redistilled water and C. P.  $\rm H_2SO_4$ , respectively, were used. Since only small amounts of chemicals are used in the preparation of these supplementary solutions, no purification was attempted in this case.

DIRECTIONS FOR PURIFICATION OF MOLAR SOLU-TIONS OF SALTS.—As already pointed out, molar stock solutions of each salt were purified separately, and the complete culture solutions were prepared by adding the requisite amounts of each nutrient salt to redistilled water. All the empirical purification procedures given below, although differing for the various salts, involve the use of calcium carbonate and heating the solution in the presence of calcium and phosphate ions at slightly alkaline reactions. The general considerations underlying the calcium carbonate purification method have been discussed by Steinberg (1935), who used it to purify dilute complete nutrient solutions. In the present work the calcium carbonate adsorption method has been modified to permit the purification of relatively concentrated solutions (molar stock solutions of individual salts).

Reagents needed: (1) CaCO<sub>3</sub> U. S. P. grade, precipitated, finely divided. (2) M/1 Ca(NO<sub>3</sub>)<sub>2</sub>. (3) M/1 K<sub>2</sub>HPO<sub>4</sub>. (4) M/1 H<sub>2</sub>SO<sub>4</sub> or M/1 HNO<sub>3</sub>, diluted from concentrated "C. P. acids, special for lead and arsenic determinations." Used for acidifying the purified molar solutions of (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub> and K<sub>2</sub>HPO<sub>4</sub>.

Molar solutions of the salts were prepared with ordinary distilled water. They were purified in five liter batches, in six-liter Pyrex boiling flasks, stoppered with a cotton plug. The purified solutions were stored in Pyrex flasks. The flasks were cleaned, prior to use, with 1-1 commercial hydrochloric acid and rinsed with distilled water.

PREPARATION OF PURIFIED SALTS.—A. Calcium nitrate or potassium nitrate.—Add 65 grams of CaCO<sub>3</sub> and 50 cc. of M/1 K<sub>2</sub>HPO<sub>4</sub> to 5 liters of a molar solution of the salt. Autoclave for one hour at 20 pounds pressure. Allow the solution to stand over night and filter through a 24 cm. folded filter paper. Test the filtrate for freedom from metals by the dithizone test described below.

B. Di-potassium phosphate or di-ammonium phosphate.—Add 65 grams of CaCO<sub>3</sub> and 25 cc. M/1 Ca(NO<sub>3</sub>)<sub>2</sub> to 5 liters of each of the molar solutions and treat as under A. The pure filtrate is acidified to pH 5.5 with the special grade H<sub>2</sub>SO<sub>4</sub> or HNO<sub>3</sub>.

C. Magnesium sulphate.—Add 65 grams of CaCO<sub>3</sub>, 50 cc. M/1 Ca(NO<sub>3</sub>)<sub>2</sub>, and 50 cc. of M/1 K<sub>2</sub>HPO<sub>4</sub> to 5 liters of a molar solution of magnesium sulphate and treat as under A.

D. Ammonium sulphate.—Treat as under C, but heat the solution for about 45 minutes in an Arnold steam chamber instead of autoclaving.

THE DITHIZONE TEST FOR TOTAL METALS.—Reagents needed: (1) Redistilled chloroform. (2) Redistilled NH<sub>4</sub>OH, approximately 6 Normal. (3) Redistilled water. (4) Redistilled HCl. (Distilled from a 6 Normal solution). (5) Dithizone reagent; dissolve 100 milligrams of diphenylthiocarbazone in 100 cc. of redistilled chloroform.-Before using the dithizone for sensitive colorimetric tests, it is desirable to purify it and also to remove such metal impurities as may adhere to the walls of the separatory funnel from previous determinations. This is carried out as follows: place 10 cc. of water and 5 cc. of chloroform (1) in the separatory funnel, add 3 drops of the dithizone dissolved in chloroform and 5 drops of the 6 N NH4OH (2). Shake vigorously and allow the chloroform layer to settle out. Remove the chloroform layer and save the dithizone contained in the ammoniacal water for the subsequent test to be made in the same separatory funnel.—(6) Purified ammonium citrate reagent, prepared as follows: Dissolve 192 grams of C. P. citric acid in 500 cc. of distilled water. Add 150 cc. of concentrated NH4OH, cool, and add additional NH<sub>4</sub>OH to give a faint pink color with phenolphthalein. Bring the volume of the prepared ammonium citrate solution to one liter. Place 500 cc. of this solution in a oneliter separatory funnel. Add 10 mg. of dithizone and 10 cc. of chloroform (1) and shake. Draw off the chloroform layer, add 10 cc. of chloroform (1) and 2 mg. portions of dithizone until enough dithizone has been added to maintain a straw-yellow color in the aqueous layer, after the solution is shaken. If the ammonium citrate solution is free from metals, the aqueous layer will be straw-yellow in color and the chloroform layer green. If a reddish coloration, indicating the presence of metals, is observed in the chloroform layer, draw off the chloroform layer again. Continue adding dithizone and extracting with chloroform until the ammonium citrate solution is free from metals as indicated by absence of any reddish tinge in the green chloroform layer.

DIRECTIONS FOR TESTING THE PURITY OF WATER. -Place 200 cc. of the water to be tested and 5 cc. of chloroform in the separatory funnel containing purified dithizone (5), shake vigorously for a minute or more, and then allow the chloroform layer to settle out. A red or purple color in the chloroform layer indicates the presence of one or more of the following metals: zinc, copper, lead, nickel, cobalt, mercury, cadmium, thallium, and bismuth. Quantitative estimates are made by comparing with standards prepared by adding known amounts of metals to pure water and treating the solution in the same manner. Known amounts of zinc were generally used in the preparation of standards. If less than 0.2 y of metals (by comparison with a zinc standard) is found in the 200 cc. sample, the water is designated as containing less than one part per billion of metals. The actual metal content may be estimated more accurately by testing a larger sample of water.

DIRECTIONS FOR TESTING THE PURITY OF MOLAR STOCK SOLUTIONS.—Molar stock solutions of all salts were approved for use if found to contain less than 10 parts per billion of metal impurities. This degree of purity was usually attained after one purification. In certain cases, with heavily con-

taminated salts, a second treatment was necessary. In such cases the filtrate obtained after the first purification was treated as the original stock solution. Directions for testing the various salts are given below:

A. Calcium nitrate, potassium nitrate, or dipotassium phosphate.—Place 50 cc. of the purified molar salt solution and 100 cc. of the tested redistilled water in the separatory funnel containing the purified dithizone (5), add 5 cc. of chloroform. shake vigorously for a minute or more, and allow the chloroform layer to settle out. A red or purple color in the chloroform layer indicates the presence of one or more of the metals previously listed. Estimate the amount of metals present by comparing the color of the chloroform layer with that of a standard, prepared by adding a known amount of a metal (zinc sulphate) to pure water and extracting with dithizone and chloroform as described above. If less than 0.5 gammas of metals is found in 50 cc. of molar salt solutions (corresponding to less than 10 parts per billion), the salt is approved for use.

B. Di-ammonium phosphate or ammonium sulphate.—In the presence of ammonium salts, dithizone is slightly soluble in the chloroform layer and imparts to it a green color which masks the usual red of the metal-dithizone complexes. It is therefore necessary after proceeding as in A to remove the green color by withdrawing the chloroform layer to another separatory funnel containing 50 cc. of tested water and 2 drops of NH<sub>4</sub>OH (2), shaking, and allowing the chloroform layer to settle out. Compare the now apparent red color of the dithizone metal complexes which remain in the chloroform layer with a standard as in A.

C. Magnesium sulphate. - Add 5 cc. of the purified ammonium citrate reagent (6) to the separatory funnel containing the purified dithizone and proceed as under B. The ammonium citrate prevents the formation of magnesium phosphate in the alkaline solution. Since ammonium salts have been added, a green color appears in the chloroform layer which is removed as in the test for ammonium phosphate or sulphate.

EXPERIMENTS WITH PLANTS.—Biological confirmation of the efficacy of the purification procedures described above was obtained from experiments with tomato plants. Plants of the Crackerjack (Earliana) variety were germinated in pure acid-washed sand and transplanted into 2-liter Pyrex beakers filled with nutrient solution. Eight plants were grown in each beaker. The composition of the nutrient solution, including the solutions A4 and B7 of supplementary microelements, was previously described (Arnon, 1938).

The complete list of microelements furnished by the A4 and B7 solutions consisted of boron, manganese, zinc, copper, molybdenum, vanadium, titanium, tungsten, chromium, nickel, and cobalt. To produce deficiency symptoms of manganese, zinc,

and copper, each of these three elements, respectively, was omitted from the complete list.

The importance of purification of water and chemicals was tested by growing plants in four series of nutrient solutions prepared as follows: (1) Ordinary distilled water and unpurified salts; (2) ordinary distilled water and purified salts;

(3) redistilled water and unpurified salts; (4) redistilled water and purified salts.

Duplicate cultures of each of the four treatments -complete, minus zinc, minus manganese, and minus copper-were used in each series, giving a total of 32 cultures.

Plants were set out in the nutrient solutions on April 11. After three weeks plants growing in the minus manganese nutrient solutions made with purified salts (series 2 and 4) developed characteristic manganese deficiency symptoms (Schreiner and Dawson, 1927). These symptoms in tomato plants bear a marked resemblance to "frenching" symptoms in tung trees, also traceable to manganese deficiency (Reuther and Dickey, 1937).

The development of manganese deficiency symptoms was confined to cultures in series 2 and 4, in which purified salts were used with distilled and redistilled water, respectively. If unpurified salts were used (series 1 and 3), no manganese deficiency symptoms were observed, whether distilled or redistilled water was used (table 2). It was evident that the unpurified salts, and not the distilled water, formed the important source of manganese impurities.

Different results were obtained with the minuszinc and minus-copper cultures. Characteristic zinc and copper deficiency symptoms (Reed, 1939) were found in three weeks old plants of series 3 and 4, in which redistilled water was used, with unpurified and purified salts, respectively, but not in series 1 and 2, in which distilled water was used The important source of copper and zinc contamination was the distilled water and not the unpurified salts. With redistilled water it was possible to produce at early growth stages copper and zinc deficiency symptoms even when unpurified salts were used in the preparation of nutrient solutions. However, an earlier onset and a greater severity of deficiency symptoms was evident in series 4 (redistilled water and purified salts), indicating that the unpurified salts were yielding some zinc and copper impurities to the solutions but in amounts not adequate for the needs of the plant.

As the plants increased in size, zinc and copper deficiency symptoms gradually developed in cultures with ordinary distilled water (series 1 and 2), suggesting that the impurities derived from this source became inadequate in supplying the increasing requirements of the plants. Manganese deficiency symptoms have likewise developed with time in minus manganese cultures, in series 1 and 3 in which unpurified salts were employed in the preparation of nutrient solutions.

Table 2. Average dry weights in grams of young tomato plants (8) grown in complete and deficient nutrient solutions of varied purity.

	Comp	Complete		Minus copper		Minus zinc		Minus manganese	
Series	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	
1. Distilled water Unpurified salts	} 2.6	0.90	2.7	0.85	2.0	0.75	2.4	0.85	
2. Distilled water Purified salts	∵} 2.9	0.90	2.5	0.85	1.2	0.75	1.8	0.50	
3. Redistilled water Unpurified salts	$\left. \begin{array}{c} \cdot \cdot \\ \cdot \cdot \\ \end{array} \right\} \;\; 2.9$	0.85	1.8	0.65	0.75	0.55	2.4	0.95	
4. Redistilled water Purified salts	•	0.95	1.1	0.55	0.45	0.55	1.7	0.50	

These and other findings not included in this report, showed that consistent demonstrations of early deficiency symptoms of zinc, copper, and manganese at all seasons of the year were possible only if redistilled water and purified salts were used. But if tomato plants were grown to relatively large size in cultures deficient in one of the three metals, deficiency symptoms were produced at times without resorting to any or with only partial purification of the nutrient medium.

The plants in the complete nutrient solutions grew equally well in the 4 series. The dry weights of plants grown for five weeks in the various nutrient solutions are given in table 2. The reduced weights of roots and shoots in the different series reflect the impairment of growth as a consequence of a particular deficiency and provide a relative measure of the importance of purification procedures for the several elements. In other experiments, by allowing the plants to grow longer, more striking differences were shown between the complete and deficient cultures, as a result of the continuously favorable growth of the former and the decline and necrosis of the latter.

The observed development of copper, manganese, and zinc deficiency symptoms were not only wholly prevented by the inclusion of these elements in the culture medium (in concentrations of 0.02, 0.5, and 0.05 ppm., respectively), but recovery and resumption of normal growth was effected by spraying the deficient cultures with dilute solutions of the elements concerned. This provided direct proof of the specific and direct relation of these elements to the nutrition of the plant quite apart from their possible effects on root environment (Arnon and Stout, 1939).

Discussion.—The results demonstrated the desirability of rigid purification of the nutrient medium for investigations on the relation of microelements to plant nutrition. The amounts of contaminants derived from containers, distilled water, and C. P. salts varied greatly and at times supplied in full plant requirements for a given microelement.

The purification procedures described give an effective and comparatively simple method for

preparation of large volumes of nutrient solutions relatively free from heavy metal impurities. The suitability of this technique for investigations on the rôle of certain microelements in the nutrition of higher plants is indicated by the fact that consistent responses were obtained from adding small amounts of zinc, copper, manganese, and certain other heavy metals such as molybdenum to deficient cultures prepared with redistilled water and purified salts. Attention is called, however, to the unsuitability of these purified salts for producing deficiencies of "macro-elements" since small amounts of calcium, potassium, nitrogen and phosphorus are incorporated in the molar stock solutions in the purification process.

The use of the sensitive dithizone test for the analytical determination of metal contaminants was distinctly helpful. It offered a means of quantitative estimation of the purity of the individual constituents of the nutrient medium before undertaking qualitative biological tests for producing deficiency symptoms in a growing plant and made it possible to prepare in Pyrex containers nutrient solutions of equal and reproducible purity.

The minute requirement of plants for elements like copper endows them in a sense with the power to serve as biological indicators for the presence of minute amounts of certain metals in distilled water or in chemicals. In the present investigation for example, distilled water alone, derived from a usual laboratory still (tin-lined copper) was found at times to supply enough copper for the growth of young tomato plants.

A distinction must be made between chemical and biological purity of water and chemicals. It is clear, considering the minute requirements of plants, that purity of chemicals, in the usual analytical sense, expressed generally in terms of percentages of impurities present, may be quite meaningless, if freedom from microelements is considered. The value of a preliminary chemical analysis of all constituents of the nutrient medium as by spectrographic means (Chapman, Vanselow, and Liebig, 1937) is evident.

The special importance of a suitable experimental technique in research on microelements adds weight to positive findings which show plant responses to addition of minute amounts of certain elements. A subsequent failure to repeat such results may mean, in the absence of evidence to the contrary, that either the culture medium became contaminated or that an impurity was added through the aerial portions of the plant. It would seem best, in experiments with metals determinable by the dithizone test, to find analytically the upper limit of impurity that may be present in the culture medium and to express the results of experiments on a quantitative basis—that is, that with a certain upper limit of supply, a given response is obtained with certain species of plants. A further discussion of this viewpoint is presented elsewhere (Arnon and Stout, 1939).

#### SUMMARY

An experimental water-culture technique adapted to ordinary laboratory and greenhouse facilities for investigating the physiological importance of zinc, manganese, and copper in the nutrition of higher plants is described.

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The technique involves (a) the selection of suitable containers, (b) purification of water by redistillation, using a Pyrex glass condenser, and (c) purification of molar stock solutions of individual nutrient salts. Adaptations of Steinberg's calcium carbonate procedure for purifying the nutrient solution as a whole were devised for the purification of the relatively concentrated molar stock solutions of individual nutrient salts.

The purity of water and each individual molar stock solution of nutrient salts is tested prior to use by means of a specially adapted dithizone test. By using Pyrex glass containers and tested water and salts, nutrient media of reproducible purity were prepared at all times.

Biological confirmation of the purity of nutrient medium was obtained by growing plants in nutrient solutions deficient in manganese, zinc, and copper, respectively. Plants consistently developed early deficiency symptoms characteristic of each element.

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# FURTHER EXPERIMENTS ON EXCISED TOMATO ROOTS 1

William J. Robbins and Mary Bartley Schmidt

We reported previously (1937a, 1937b, 1938) that excised tomato roots require for unlimited growth an external supply of thiamin (vitamin  $B_1$ ) or the vitamin thiazole (4-methyl-5- $\beta$ hydroxy-ethyl thiazole), in addition to sugar, mineral salts, and water. We have maintained excised tomato roots in a solution of mineral salts, pure cane sugar, and thiamin through 25 successive passages extending over a period of more than two years. In this solution the average growth in a period of two months of a single root rarely exceeds 10 mg. and is usually less. However, when a light brown sugar is

<sup>1</sup> Received for publication January 19, 1939. Supported in part by a grant from the American Philosophical Society. substituted for the pure cane sugar in the solution given above, the growth is much improved, the average dry weight of individual roots reaching 70 or 80 mg. (fig. 1).

Why is the brown sugar beneficial? We believe that it is in part because of the minerals contained in the sugar, but primarily because of the presence of vitamin B<sub>6</sub>. We present evidence in this paper that in the presence of thiamin this portion of the B complex has a marked beneficial effect upon the growth of excised tomato roots (see also Robbins and Schmidt, 1939).

METHODS AND MATERIALS.—The methods used in this investigation are similar to those described earlier (Robbins and Schmidt, 1938). Excised to-

mato roots were grown individually in 50 ml. of solution in 125 ml. Erlenmeyer flasks of Pyrex glass. The roots came originally from seeds of a pink fruited variety of *Lycopersicon esculentum* from Mexico. The excised roots have been kept in cultivation since September 29, 1935. They represent two clones which respond alike.

The nutrient solutions used had the following

composition per liter:

White's mineral solution: Ca(NO<sub>3</sub>)<sub>2</sub>, 0.142 g.; MgSO<sub>4</sub>7 H<sub>2</sub>O, 0.073 g.; KNO<sub>3</sub>, 0.081 g.; KCl 0.065 g.; KH<sub>2</sub>PO<sub>4</sub>, 0.012 g.; Fe<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub>, 0.0024 g.

Pfeffer's mineral solution (modified): Ca(NO<sub>3</sub>)<sub>2</sub>, 0.333 g.; MgSO<sub>4</sub>7 H<sub>2</sub>O, 0.063 g.; KNO<sub>3</sub>, 0.063 g.;

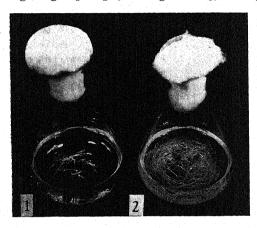


Fig. 1. Effect of light brown sugar. 1, root grown in Pfeffer's solution plus  $10\,\gamma$  of thiamin and 2 per cent pure cane sugar; 2, in same solution with 2 per cent light brown sugar instead of pure cane sugar. Age about 2 months.

KCl, 0.042 g.;  $KH_2PO_4$ , 0.060 g.;  $Fe_2(SO_4)_3$ , 0.0025 g.

Mineral solution W: White's mineral solution

plus 0.1 ppm. each of zinc and of boron.

The mineral salts were of chemically pure grade and not especially purified. The cane sugar was Pfanstiehl's C. P. sucrose. The thiamin was Merck's synthetic vitamin  $B_1$  (Betabion). The solutions were sterilized in an autoclave at 10 or 15 pounds steam pressure. The roots were grown in diffuse light at temperatures ranging from  $20^{\circ}$  to  $25^{\circ}$ C.

EXPERIMENTAL RESULTS.—Effect of brown sugar.

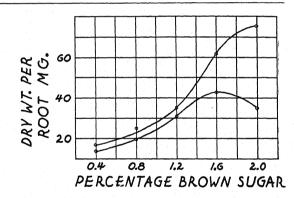
—The beneficial effect of brown sugar as compared with pure cane sugar was reported earlier (Robbins and Schmidt, 1938). We have used six samples of light brown sugar and have found all of them beneficial, though not equally so. The growth of the excised roots in White's mineral solution or Pfeffer's mineral solution plus 2 per cent brown sugar and thiamin is slow in starting, and the roots are long, white, with short, sparse root hairs. Growth continues for three months or more, and individual roots weighing 165.0 mg. have been obtained. Dark brown sugars at 2 per cent are sufficiently injurious

to inhibit growth entirely. In fact the light brown sugars also at 2 per cent concentration are somewhat injurious, causing, as we believe, the slow starting of growth and the partial inhibition of growth of some roots noted in several instances.

Dilution of brown sugar.—When the light brown sugars are diluted, the total growth is decreased, as illustrated in the following experiment: White's mineral solution plus 2 per cent light brown sugar No. 5 and 0.2 ppm. of thiamin was diluted with White's mineral solution plus 2 per cent pure cane sugar and 0.2 ppm. of thiamin to produce solutions

Table 1. Effect of dilution of light brown sugar in White's mineral solution, sugar, and  $10\,\gamma$  thiamin per flask on growth of excised tomato roots. Roots grown two months at  $20^\circ-25$  C. Total concentration of sugar maintained at 2 per cent by addition of pure cane sugar. Passage 33.

Percentage light brown sugar	No. roots weighed		Range of dry wts., mg.	Av. dry wt two best roots, mg.
2	5	34.6	4.5-72.6	72
1.6	5	41.6	21.6-65.7	60.4
1.2	4	30.0	24.5-36.5	34.6
0.8	5	19.3	14.6-29.4	24.7
0.4	5	13.5	8.6-19.2	16.4
None	5	3.5	0.4-6.9	



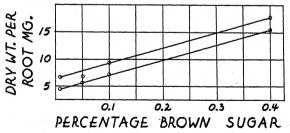


Fig. 2 (above). Effect on growth of excised tomato roots of dilution of light brown sugar, total concentration of sugar 2 per cent by addition of pure cane sugar. Lower curve average of 5 roots, upper curve, average of 2 best roots. Age about 2 months.

Fig. 3 (below). Effect on growth of excised tomato roots of dilution of two samples of light brown sugar. Total concentration of sugar 2 per cent by addition of pure cane sugar. Age about 2 months.

containing 2, 1.6, 1.2, 0.8, 0.4 per cent, and no light brown sugar. The total concentration of sugar in each of these solutions was 2 per cent. Five replications for each solution were used in the experiment which was run for two months. The results are presented in table 1 and figure 2.

When the average of all the roots grown at a particular concentration is considered, the optimum for this sample of brown sugar was 1.6 per cent. This is because of the greater variation in growth at the higher concentrations of brown sugar where some injurious factor or factors (probably the ash) inhibited some of the root tips. If the average of the two best roots is considered, a dilution of the sugar to 1.6 per cent decreased the growth. This suggests that the amount of the beneficial factor or factors in the 2 per cent light brown sugar does not exceed the optimum.

Dilution of the brown sugar below 0.4 per cent reduced growth further, as is shown in table 2 and

Table 2. Effect of dilution of two samples of light brown sugar in White's mineral solution plus  $10\gamma$  of thiamin per flask. Roots grown two months at  $20^{\circ}-25$  C. Total concentration of sugar maintained at 2 per cent by addition of pure cane sugar. Passage 33.

Percentage light grown sugar	No. roots weighed	Av. dry wt. per root, mg.	Range of dry wts., mg.
Sample No. 5			
0.4	5	15.5	4.0-23.4
0.1	5	7.2	3.8-10.7
0.05	5	5.8	2.0- 9.0
0.01	5	4.7	2.3- 6.1
Sample No. 6			
0.4	5	17.9	7.5-27.3
0.1	5	9.2	7.9-11.6
0.05	5	6.9	3.2- 9.6
0.01	5	6.6	1.7–12.8

figure 3. Two samples of brown sugar were used in this experiment, No. 5 and No. 6, and each was used at 0.4, 0.1, 0.5, and 0.01 per cent; the total concentration of sugar was made 2 per cent by the addition of pure cane sugar. From the data in table 2 and the curves in figure 3, sample No. 6 appears to contain more of the beneficial material than sample No. 5. The relation between amount of brown sugar and growth at these greater dilutions was linear.

Effect of ash of light brown sugar.—In discussing the highly beneficial effect of the light brown sugar, we reported (1938) that its ash, in amounts equivalent to that in a 2 per cent solution of the sugar, proved toxic but suggested that smaller amounts of the ash might be beneficial. The possibility that the favorable action of the brown sugar might be associated with the minerals it contains has been investigated further, but we have been unable to demonstrate that the ash produces more than a minor benefit. Furthermore, the growth of tomato

roots in 2 per cent light brown sugar and thiamin to which no mineral salts were added was slight in spite of the relatively high ash content of the sugar. This is probably because of a deficiency of nitrates.

Pfeffer's mineral solution plus 2 per cent pure cane sugar and 10 y of thiamin per flask was supplemented with amounts of the ash of light brown sugar equivalent to that contained in 2, 1, 0.4, and 0.2 per cent solutions of the sugar. This experiment was repeated using ash which had been treated with HCl. To 160 mg. of the ash 10 ml. of HCl were added and heated to dryness, the procedure was repeated, and the residue taken up in water. Little benefit was observed from the untreated ash at any of the concentrations (table 3). In fact at an amount equivalent to that in a 2 per cent solution of sugar the ash inhibited growth entirely and was somewhat injurious at one-half that strength. Some benefit was derived from the treated ash at concentrations equivalent to the ash in 1, 0.4, and 0.2 per cent sugar solutions, the most favorable result appearing at 0.4 per cent. However, even here the growth was much less than that obtained with brown sugar. It is recognized that some minerals are volatilized to a greater or less extent in the ashing and some are changed in form. We are not certain whether the treated ash was more favorable than the untreated ash because of further volatilization of some elements, because the HCl brought some beneficial elements into solution, or because something was added in the HCl used in the digestion. In any event, the experiments with the ash of brown sugar did not encourage us to look there for the favorable factor of primary importance. We therefore turned our attention to the organic constituents of the impure sugar.

Again, as in our investigation which resulted in our discovery of the importance of thiamin for the growth of excised tomato roots, there were two obvious means of approach to this problem. One was to fractionate the sugar in various ways to find some clue to the favorable factor or factors, the other was to try as many suspected substances as might be available. Both procedures were followed.

Extraction of light brown sugar.—Attempts to extract the beneficial material by the use of various solvents and adsorption on "decalso" were not particularly successful. In several instances some benefit resulted from the addition of the extracts to Pfeffer's solution containing pure cane sugar and thiamin, but in none was the growth comparable to that in solutions containing the light brown sugar. It would doubtless be entirely possible to prepare extracts which contained the beneficial materials, but time and facilities limited our efforts in that direction.

A number of substances including heteroauxin, lactoflavin, cysteine hydrochloride, and pimelic acid were previously used to supplement a mineral solution containing cane sugar and thiamin (Robbins and Schmidt, 1938). None of them, as we used

them, improved the growth of excised tomato roots. In addition, we have tried nicotinic acid, nicotinamide, amino acids, and vitamin  $B_6$ .

Nicotinic acid and nicotinamide.—Knight (1937a, 1937b) found nicotinic acid or nicotinamide necessary for the growth of Staphylococcus aureus. We found excised tomato roots made little growth in Pfeffer's mineral solution plus 2 per cent pure cane sugar and  $10 \gamma$  per flask of nicotinic acid or nicotinamide. The growth in the same solutions to which  $10 \gamma$  of thiamin per flask were added was about the same as that in the Pfeffer's solution plus cane sugar and thiamin alone. We concluded that nicotinic acid or nicotinamide did not substitute for thiamin in the growth of tomato roots and was not the factor chiefly responsible for the favorable action of brown sugar.

Amino acids.—White (1937a, 1937b) in studies of the separation from yeast of materials essential for the growth of excised tomato roots concluded that an important part of the yeast fraction soluble in 85 per cent alcohol was amino acids. He prepared a mixture of amino acids which when added to a solution of sugar and mineral salts exerted a favorable effect on the growth of the roots. When the amino acid mixture and a yeast fraction soluble in 100 per cent alcohol were used by White as supplements to a mineral salt-sugar solution, growth

Table 3. Growth of tomato roots in Pfeffer's solution plus 2 per cent sugar and 10 \gamma of thiamin per flask supplemented with ash of light brown sugar or same ash digested with HCl. Period of growth two months. Passage 33.

		Untrea	ted ash	Treated ash			
	sh equiv. o sugar of	No. roots weighed	Av. dry wt. per root, mg.	No. roots weighed			
	2%	5	0.2	5	3.2		
	1%	5	2.1	5	9.2		
	0.4%	5	3.4	5	9.9		
	0.2%	5	5.0	5	7.9		
	Check	5	3.5		3.5		
. 4							

approached that in a solution supplemented with yeast. In a later paper White (1937c) concluded that thiamin was a constituent of the yeast fraction soluble in 100 per cent alcohol important for the growth of excised tomato roots.

In our experiments an absolute alcohol extract of yeast was ineffective (1937a, 1938). In fact, thiamin is extremely sparingly soluble in neutral absolute alcohol and is not extracted from yeast and food stuffs by this solvent unless an excess of mineral acid is present (Williams, 1937). Furthermore, we have been unable to detect a favorable effect of White's amino acid mixture on the growth of our strain of tomato roots in a mineral-sugar solution, or in a mineral-sugar solution supplemented with thiamin.

The provisional amino acid mixture per liter given by White as "complete and possibly optimal" and the source of our amino acids were as follows: 5 mg. d- glutamic acid, Eastman; 1.5 mg. dl- phenyl alanine, Marvel; 1.5 mg. dl- lysine, Marvel; 1.5 mg. l- histidine, Eastman; 1.5 mg. l- proline, Pfanstiehl; 0.15 mg. dl- valine, Marvel; 0.05 mg. dl-serine, Pfanstiehl; 0.015 mg. dl-norleucine, Marvel; 0.0015 mg. dl-isoleucine, Marvel.

In our experiments the addition of this mixture of amino acids to White's mineral solution plus 2 per cent sugar and  $10\,\gamma$  of thiamin per flask had no effect (table 4), nor was any effect noted when mineral solution W plus cane sugar and thiamin was used. The addition of the amino acid mixture to these solutions was not injurious, the roots grew, but grew as well without the amino acids as with them. No growth was secured in mineral solution W plus 2 per cent cane sugar and the amino acid mixture. The amino acid mixture was not found to substitute for nitrates. When the nitrates in White's solution were replaced with the amino acid mixture at the concentration given or at 5 times that strength, no growth occurred.

We concluded that under the conditions of our experiments the amino acid mixture was of no significant benefit and that the beneficial effect of light brown sugar could not be credited to its possible amino acid content. Bonner and Addicott

Table 4. Effect of an amino acid mixture on growth of excised tomato roots in solution W or in White's solution plus 2 per cent cane sugar and 10  $\gamma$  thiamin per flask. Inoculum for all but last two sets from roots grown in solution W + thiamin; last two sets inoculated from roots grown in a 2 per cent maltose solution.

Passage Solution		No. roots weighed	Av. dry wt. per root, mg.	Range of dry wts., mg.
No. 25 W + thiamin	cids	5	5.0 4.5 0.6	0.2-11.2 2.7-10.7 0.1- 1.1
No. 26 White's + thiamin White's + thiamin and ar W + thiamin White's + thiamin and ar	nino acids	5 9 5 5	8.3 8.2 10.3 10.6	0.7-13.4 0.2-22.7 1.7-25.1 1.6-23.1

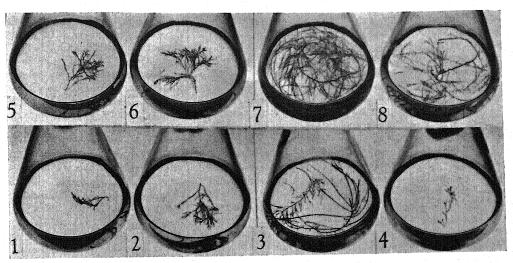


Fig. 4. Effect of vitamin  $B_6$  on growth of excised tomato roots in 1, Pfeffer's solution plus 2 per cent cane sugar; 2, same plus  $5\gamma$  thiamin; 3, plus  $5\gamma$  thiamin and  $1\gamma$  vitamin  $B_6$ ; 4, plus  $1\gamma$  vitamin  $B_6$ ; 5, Pfeffer's solution plus 2 percent cane sugar and  $5\gamma$  thiamin; 6, same plus  $5\gamma$  thiamin and light brown sugar ash; 7, plus  $5\gamma$  thiamin,  $5\gamma$  vitamin  $B_6$  and ash; 8, plus  $5\gamma$  thiamin and  $5\gamma$  vitamin  $B_6$ . Age about 2 months.

(1937) reported amino acids to be beneficial to the growth of excised pea roots. Pea roots and the strain of tomato roots used by White may respond differently from the roots we have used. We recognize also that amino acids may prove beneficial to the growth of our strain of tomato roots when other limiting factors have been removed. However, the roots we have used were capable of constructing the necessary proteins from nitrates, as is evidenced from the results of our experiments in which nitrates have been the sole source of nitrogen.

Vitamin  $B_6$ .—Emerson, Mohammad, Emerson, and Evans (1938) reported that molasses is rich in vitamin  $B_6$ . Although this vitamin has not hitherto been demonstrated to be important in plant growth, its presence in molasses and its probable presence in brown sugar suggested its use. Kuhn and Went (1938), Lepovsky (1938), and Keresztesy and Stevens (1938) reported its isolation in crystalline form. Through the courtesy of Merck and Co., a sample of crystalline vitamin  $B_6$  isolated from natural sources was secured. Its empirical formula

as given by Merck and Co. is  $C_8H_{11}NO_3$ .HCl. Vitamin  $B_6$  produced a marked increase in the growth of excised tomato roots when added to a solution of mineral salts, pure cane sugar, and thiamin. In fact, the growth in mineral-sugar solutions supplemented with both thiamin and vitamin  $B_6$  materially surpassed that in a mineral-sugar-yeast solution. Furthermore, the addition of vitamin  $B_6$  to diluted light brown sugar solutions supplemented with thiamin markedly increased the growth, demonstrating that in such solutions vitamin  $B_6$  was the limiting factor.

In the first experiment with vitamin  $B_6$  the excised roots were grown for two months in Pfeffer's solution containing 2 per cent pure cane sugar supplemented with thiamin, vitamin  $B_6$ , and light brown sugar ash, which had been treated with HCl. The ash was used at a concentration equivalent to that in a 0.4 per cent sugar solution. Thiamin was added at the rate of 5  $\gamma$  per flask, and vitamin  $B_6$  at 1  $\gamma$  and 5  $\gamma$  per flask (table 5). Vitamin  $B_6$  used alone in Pfeffer's solution containing pure cane

Table 5. Effect of thiamin, vitamin  $B_6$ , and ash of light brown sugar on growth of roots in Pfeffer's solution plus 2 per cent pure cane sugar. Roots grown two months. Passage 34.

Pfeffer's solution containing 2% pure cane sugar plus			Range of dry wts., mg.	Av. dry wt. two best roots, mg.	
	5	0.4	0.1- 1.5	0.9	
No addition	5	3.4	1.4- 4.7	4.6	
5γthiamin	5	16.1	12.9-22.0	20.4	
$5 \gamma$ thiamin and $1 \gamma$ vitamin $B_6 \ldots$	5	15.7	14.4-16.3	16.2	
$5 \gamma$ thiamin and $5 \gamma$ vitamin $B_6 \ldots$	5	1.8	0.1-8.4	4.3	
iγvitamin B <sub>6</sub>	. 5	1.3	0.4- 3.2	2.2	
$5 \gamma$ vitamin $B_6 \ldots \ldots$	. ,	7.6	1.2- 9.9	9.5	
γ thiamin plus ash		14.5	3.7-25.3	23.2	
5 $\gamma$ thiamin plus 1 $\gamma$ vitamin $B_6$ and asl 5 $\gamma$ thiamin plus 5 $\gamma$ vitamin $B_6$ and asl	n 5 h 5	22.3	8.8-34.3	30,3	

sugar had little effect on growth,2 but in the presence of thiamin the benefit was noticeable within the first week of growth. The roots in the solutions containing thiamin and vitamin B6 grew more rapidly than in the solutions supplemented with thiamin alone and developed a particularly luxuriant crop of root hairs. The growth was more rapid in the solutions supplemented with thiamin and 5 y of vitamin B<sub>6</sub> than in those supplemented with thiamin and 1 y of B6, although the final dry weights (table 5) taken at the end of two months did not show this difference except where the solution contained the light brown sugar ash. Evidently some factor, probably the mineral supply, limited the total growth in the Pfeffer's solution not supplemented with ash. At any rate, the growth in the solutions containing thiamin and vitamin B6 was several times that in the solutions containing thiamin only.

Amount of vitamin B<sub>6</sub> and growth.—In the early stages of the above experiment the roots grew more rapidly in those solutions containing 5  $\gamma$  of vitamin B<sub>6</sub> than in those containing 1 γ, though the difference in growth (except where ash was added also) did not appear in the final dry weights. Two experiments were performed with various amounts of vitamin B<sub>6</sub> per flask. In the first, amounts from 0.000 000 1 y to 10 y per flask in multiples of ten were added to Pfeffer's solution containing 2 per cent pure cane sugar and 10 y of thiamin per flask. The roots were grown for two months. No benefit was noted from the addition of amounts of vitamin  $B_6$  from 0.000 000 1  $\gamma$  up to and including 0.1  $\gamma$  per flask. The final growth with  $1 \gamma$  of vitamin  $B_6$  was about one and one-half times the growth in the Pfeffer's solution supplemented with thiamin alone, and with 10 y of B<sub>6</sub> per flask over twice as much growth was obtained.

Since there was some evidence that  $10 \gamma$  of vitamin  $B_6$  per flask was superior to  $1 \gamma$ , a second experiment was performed in which  $10 \gamma$ ,  $50 \gamma$ , and  $100 \gamma$  per flask were added to Pfeffer's solution con-

 $^2$  One root grew considerably (8.4 mg.) in the solution containing 1  $\gamma$  of vitamin  $B_6$ ; none of the others grew appreciably. We believe that sufficient thiamin was contained in the root tip to permit the growth noted, or an error in experimental procedure was responsible for this result.

taining 2 per cent pure cane sugar and 0.2 ppm. of thiamin. The roots grew more rapidly in the solutions containing  $50 \gamma$  per flask than in those containing  $10 \gamma$  or  $100 \gamma$ .

During the second week of growth, sketches were made at regular intervals of the main tip of one root in the solutions containing 50 y of vitamin B<sub>6</sub> per flask. It was found to be growing at the rate of between 0.75 cm. and 1.0 cm. per 24 hours. Fifteen days after the initiation of the experiment, growth of this tip ceased. Final dry weights were determined at the end of two months (table 6). Growth was greater in the solutions containing 50  $\gamma$  of vitamin B<sub>6</sub> than in those containing 10  $\gamma$ or 100 y. However, in this experiment the growth with 10 y was no better than in the earlier experiment with 1 y. If dry weights had been determined at the end of two or three weeks, greater differences in growth with various amounts of vitamin B<sub>6</sub> would probably have been found. It would seem that greater amounts of vitamin B6 than of thiamin are necessary in the growth of tomato roots, as responses have been secured to thiamin at amounts which were ineffective when vitamin B<sub>6</sub> was used.

Mention has already been made of the profuse root hair production in solutions containing vitamin  $B_6$ . It was observed also that in the vitamin  $B_6$  solutions portions of many of the roots eventually developed a chocolate brown color. Furthermore, a development of hooks and curls by the root tips was observed in solutions containing vitamin  $B_6$ , a condition which we have never noted in any other solution.

Growth abnormalities in solutions containing vitamin  $B_6$ .—The tomato roots in the solutions supplemented with vitamin  $B_6$  grew more rapidly than those in the solutions lacking the vitamin. However, many of the main root tips and many branch ends developed hooks, curls, and corkscrews after ten days or two weeks of growth. It was thought this might be a response to the exposure to light, and the roots were transferred to a darkened chamber where the condition disappeared.

However, we doubt whether light was concerned, as roots grown in the presence of vitamin  $B_6$  in a darkened chamber developed similar abnormalities which disappeared in time. Quintuplicate cultures were prepared in Pfeffer's solution containing 2 per

Table 6. Effect of amount of vitamin  $B_6$  on growth of tomato roots in Pfeffer's solution plus 2 per cent cane sugar and 0.2 ppm. thiamin. Above, passage 34; below, passage 36.

Addition to Pfeffer's solution plus 2% sugar and 0.2 ppm. thiamin		Av. dry wt. per root, mg.	Range in dry wts., mg.	Av. dry wt. two best roots, mg.
None	5	6.4	2.3- 9.9	9.7
$0.1\gamma\mathrm{B_6}$		7.6	3.1- 9.5	9.4
1 γ Β <sub>6</sub>	4	13.3	10.3-19.4	18.0
$10 \gamma \tilde{B}_6$		18.9	8.5-35.0	33.0
10 γ Β <sub>6</sub>	5	13.7	9.3-22.6	17.9
50 γ B <sub>6</sub>	5	20.5	6.8-31.2	31.2
$100 \gamma  \mathrm{B}_6  \dots \dots$		16.5	6.3-28.2	27.5

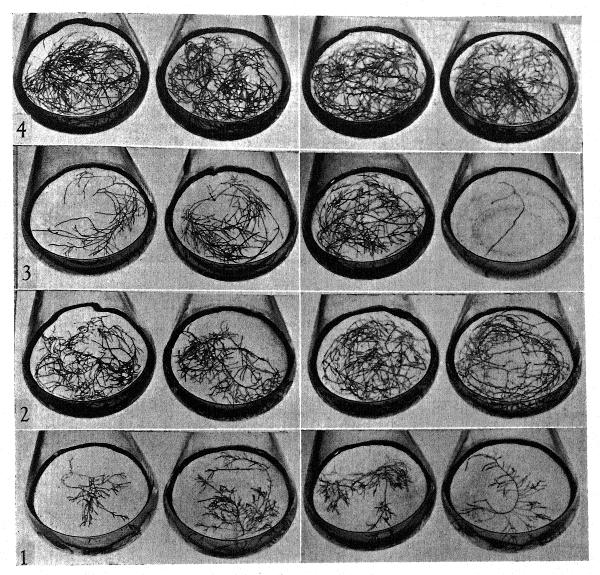


Fig. 5. Effect on growth of excised tomato roots of addition of vitamin  $B_6$  to dilute light brown sugar. 1, growth in Pfeffer's solution plus 0.4 per cent light brown sugar, 1.6 per cent pure cane sugar and 0.2 ppm. thiamin; 2, same plus  $10 \gamma$  vitamin  $B_6$  per flask; 3, in Pfeffer's solution plus 1.2 per cent light brown sugar, 0.8 per cent pure cane sugar and 0.2 ppm. thiamin; 4, same plus  $10 \gamma$  vitamin  $B_6$  per flask. Age about 2 months.

cent pure cane sugar and 0.2 ppm. of thiamin, 2 per cent pure cane sugar plus 0.2 ppm. of thiamin and 0.2 ppm. of vitamin  $B_6$  and set in the light. A similar set was placed in a dark box. This procedure was repeated with the pure cane sugar replaced by 0.4 per cent light brown sugar and 1.6 per cent pure cane sugar. The ends of branches of the roots in the solutions containing vitamin  $B_6$  developed curls and hooks, both in the light and in the dark. After about three weeks the hooks and curls were less noticeable. It appeared that the hooks and curls developed in response to the vitamin  $B_6$ , and that light was not concerned, though of course the roots in the darkened chamber were exposed to light when

examined at intervals, and we cannot say that light was entirely excluded.

Microscopic examination<sup>3</sup> of some of the curled roots demonstrated that the bending was the result of a failure of the cells on one side of the root to elongate. The cells on the concave side of a curve were about the same length as those near the apical meristem; the length of those on the convex side averaged about 5 times that of those on the concave side. Branch roots developed on the convex side of the curve, and it appeared from the observations made that with the initiation of a branch root the cells on the concave side of the curve lengthened,

<sup>3</sup> These microscopic studies were made with the assistance of Dr. Virgene W. Kavanagh.

with the result that the sharp curve disappeared, the root tending to straighten out.

Although these observations are incomplete and fragmentary, they suggest that excised tomato roots grow so rapidly in solutions containing both thiamin and vitamin B<sub>6</sub> that some substance necessary for the lengthening of the cells in the region of elongation comes near exhaustion. With the exhaustion of this material, elongation is reduced, and new quantities of the material formed in the root are unequally distributed, resulting in the formation of hooks, curls, and corkscrews. If these assumptions are correct, the substance concerned would not be thiamin, vitamin B6, or nicotinamide, since we have noted the development of these abnormalities in a solution supplemented with all three of these substances. What the material concerned is, where it is produced in the root, and why it is unequally distributed, are obvious unanswered questions.

Addition of vitamin  $B_6$  to dilute light brown sugar.—If the beneficial effect of the light brown sugar is chiefly because of its vitamin  $B_6$  content, then the decreased growth noted when the sugar was diluted, as described above, might be assumed to result from the decreased amount of vitamin  $B_6$  available. The addition of vitamin  $B_6$  to such dilute brown sugar solutions should increase the growth. We have found that this occurred.

Pfeffer's solution containing 2 per cent light brown sugar and 0.2 ppm. of thiamin was mixed with Pfeffer's solution containing 2 per cent pure cane sugar and 0.2 ppm. of thiamin to produce solutions with 0.4 per cent and 1.2 per cent light brown sugar, respectively. To some of the flasks  $10 \gamma$  of vitamin  $B_6$  were added. The growth in the solutions containing dilute brown sugar supplemented with vitamin  $B_6$  (table 7) approached that in the 2 per cent light brown sugar (table 1) which supported the conclusion that vitamin  $B_6$  was the factor primarily responsible for the beneficial effect of light brown sugar as compared to pure cane sugar.

We are of the opinion that factors other than vitamin  $B_6$  (the minerals and perhaps other growth substances) play a part in determining the beneficial action of the brown sugar. This follows because the growth in Pfeffer's solution containing 2 per cent pure sugar, thiamin, and vitamin  $B_6$ , was not as great as that in the same solution in which part of the sugar was supplied as light brown sugar.

This opinion is supported also by the difference in the growth of root tips from a solution of minerals, maltose, and yeast and those from a solution of minerals, cane sugar, and thiazole, when both are grown in solutions containing thiamin and vitamin B<sub>6</sub>. We have previously reported that some samples of maltose are more beneficial for the growth of excised tomato roots than pure cane sugar, and we were of the opinion that the difference in the effects of the two sugars was because of impurities in the maltose. Stock cultures of tomato roots in White's or Pfeffer's mineral solution plus 2 per cent maltose and yeast have been maintained as a common source of inoculum in our experiments. However, the growth of root tips from a maltose solution was found to be considerably superior to the growth of tips from roots which had been maintained for many passages in a solution of minerals, sugar, and thiazole. Since the solutions in which this difference in growth was noted contained thiamin, vitamin B<sub>6</sub>, and nicotinamide, it seemed justifiable to assume that something other than these substances was carried over in the root fragment used as inoculum and was responsible for the difference in growth. Further investigations are necessary to determine whether the "carryover" effect from the maltose solutions was due to an unknown growth substance and, if so, the nature of the sub-

These general statements are illustrated by the following experiment:

To Pfeffer's solution containing 2 per cent of cane sugar and mineral supplements we added (1)  $10 \gamma$  of thiamin per flask; (2)  $10 \gamma$  of thiamin and  $10 \gamma$  vitamin  $B_6$ ; (3)  $10 \gamma$  thiamin and  $10 \gamma$  nicotinamide; (4)  $10 \gamma$  thiamin,  $10 \gamma$  vitamin  $B_6$ , and  $10 \gamma$  nicotinamide; (5)  $10 \gamma$  vitamin  $B_6$  and  $10 \gamma$  nicotinamide. Flasks were inoculated in quintuplicate with inoculum from roots grown in Pfeffer's solution plus yeast and 2 per cent maltose. The experiment was repeated using inoculum from roots which had grown through 19 successive passages in Pfeffer's solution plus thiazole and 2 per cent cane sugar.

The dry weights of the roots at the end of one month are given in table 8. For both types of inoculum the necessity of thiamin for growth was shown by the lack of development in solutions con-

<sup>4</sup> The mineral supplements included 0.01 ppm. B, 0.02 ppm. Mo, 0.18 ppm. Zn, 0.04 ppm. Cu and 0.02 ppm. Mn.

Table 7. Effect of addition of 10 γ vitamin B<sub>6</sub> per flask on growth of roots in Pfeffer's solution containing mixtures of light brown sugar and pure sugar plus 0.2 ppm. thiamin. Total concentration of sugar 2 per cent. Grown two months. Passage 34. Compare with

Percentage light brown sugar + thiamin	No. roots weighed	Av. dry wt. per root, mg.	Range of dry wts., mg.	Av. dry wt. two best roots, mg.
Light brown sugar 0.4%	. 4	13.9	9.3-21.8	17.9
Light brown sugar $0.4\% + B_6$	. 5	33.1	25.3-47.0	42.5
Light brown sugar 1.2%		16.8	1.1-31.2	28.6
Light brown sugar $1.2\% + B_6$	. 5	46.3	39.9-49.7	49.6

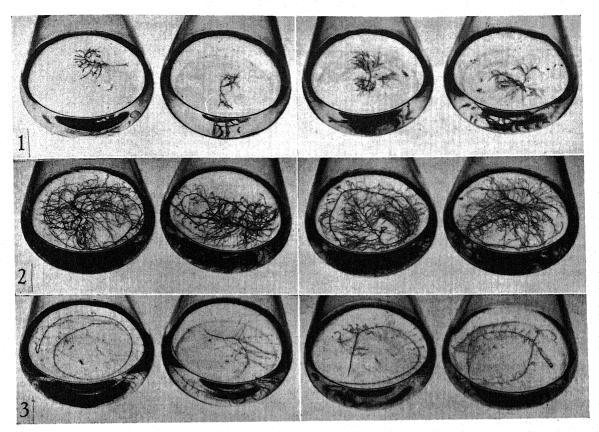


Fig. 6. Source of inoculum and effect of vitamin  $B_6$ . 1, growth in Pfeffer's solution plus 2 per cent cane sugar and 0.2 ppm. thiamin; 2 and 3, in some solution plus  $10\,\gamma$  vitamin  $B_6$  per flask. 1 and 2 inoculated with fragments of roots grown in Pfeffer's solution containing maltose and yeast; 3, inoculated with fragments of roots grown in Pfeffer's solution containing cane sugar and thiazole. Age 3 weeks.

taining vitamin  $B_6$  and nicotinamide but no thiamin. Nicotinamide had little or no effect in the presence of thiamin or of thiamin and vitamin  $B_6$ . The roots grew in all solutions which contained thiamin, but the growth was greatly increased in those solutions which contained vitamin  $B_6$  in addition. A comparison of the growth of the fragments from the roots which had grown for several passages in a solution of minerals, sugar, and thiazole with that

of fragments from roots which had grown in a solution of minerals, maltose, and yeast shows the "carryover" effect from the maltose-yeast solutions which suggests the existence of a growth factor other than thiamin, vitamin  $B_6$ , or nicotinamide.

DISCUSSION.—We are gradually gaining a clearer picture of the nutritional requirements of tomato roots and incidentally of the importance of growth substances for the development of plants. Under

Table 8. Growth of excised tomato roots in Pfeffer's solution containing mineral supplements and thiamin, vitamin  $B_6$ , or nicotinamide. Growth substances used at 10  $\gamma$  per flask. Age one month.

	Inoculum from cultures	Inoculum from maltose and yeast cultures			
Pfeffer's solution plus mineral supplements and	Av. dry No. roots wt. per weighed root, mg.	Range dry wts., mg.	No. roots weighed	Av. dry wt. per root, mg.	Range dry wts., mg.
Thiamin Thiamin and vitamin B <sub>6</sub> Thiamin and nicotinamide	5 9.9	0.3- 3.6 6.1-11.4 1.2- 7.8	. 5 5 5	5.4 25.9 5.9	3.4- 6.9 15.0-30.4 1.4-10.4
Thiamin, vitamin B <sub>6</sub> and nicotinamide	5 10.2 5 0.1	6.1–12.3 0.1– 0.2	4. 5	11.4 1.0	4.9-26.9 0.7- 1.8

the conditions of our experiments excised tomato roots synthesize little or no thiamin from the sugar and mineral salts in the medium, chiefly because of their inability to construct the intermediate thiazole; the consequence is that the excised roots are unable to grow unless furnished with thiamin or with the vitamin thiazole (Robbins and Schmidt, 1938).

However, even when supplied with more than adequate amounts of mineral salts, sugar, and thiamin, the rapidity of growth of the roots appears to be distinctly limited by their synthesis of a second vitamin, vitamin  $B_6$ . We assume that in solutions of mineral salts, sugar, and thiamin the roots synthesize vitamin  $B_6$  in amounts sufficient to permit slow growth, but not in quantity sufficient for rapid growth. The situation is somewhat analogous to the ability of  $Pythium\ Butleri$  to form sufficient thiamin for slow growth (in certain media) and its response by increased growth to additions of thiamin (Robbins and Kavanagh, 1938).

The solution of mineral salts, sugar, and yeast which we have used for the cultivation of excised tomato roots furnishes both thiamin and vitamin  $B_6$ , and the amount of the latter is probably the factor which limits growth in a solution supplemented with yeast. This follows because the growth in a solution of mineral salts and sugar supplemented with thiamin does not exceed that in a solution supplemented with yeast, even when the thiamin is present in excess, while in a solution of mineral salts, sugar, thiamin, and vitamin  $B_6$  the growth is several times that in the yeast solutions.

The function of vitamin  $B_6$  in the plant is not known. A deficiency of this vitamin interferes with the growth of rats and causes them to develop an acrodynia type of dermatitis. It is found in wheat germ autolysate, and molasses is a rich source. It was probably first isolated from rice polishings by Ohdake in 1932 (Wiarde, 1938).

The demonstration of the importance of vitamins in the physiology of plants raises questions of nomenclature which have been discussed in part elsewhere (Robbins, 1938). Since the term vitamin was limited to animals in its original definition, and the same chemical substance may be a vitamin for one organism, a hormone for a second, and neither a vitamin nor a hormone for a third, we have preferred to use the general term, growth substance. Furthermore, the demonstration that thiamin (vitamin  $B_1$ ) is important for the normal development of plants as well as animals has led us to prefer the name thiamin to that of aneurin which emphasizes the relation of that vitamin to one aspect of its importance for animals. Similarly, since vitamin B<sub>6</sub> is important for plants as well as animals, it seems

preferable to name it from its chemical constitution, when that is known, rather than to call it "adermin" as has been initiated and thus emphasize its relation to one aspect of its function in animals (Wiarde, 1938).

These observations on the importance of vitamin Bo for the growth of tomato roots add to our ideas of the dependence of the root upon the shoot, though it would be a mistake to generalize for all roots on the basis of our findings for tomato roots. We believe that many growth substances are necessary for the normal development of an organism. Some organisms synthesize all the growth substances in amounts adequate for normal growth. Others lack the ability to make one or more than one, and the synthetic ability of one organism may be different from that of another. To conclude that the growth substances which must be supplied excised tomato roots are also those which must be supplied excised corn roots, or other roots, would be in error. Furthermore, our experiments with tomato roots have been carried on in liquid cultures where the oxygen supply is limited; we cannot be certain that the responses of roots under these conditions are identical with those of roots under normal growing conditions in the soil. It seems probable, however, that the tomato root depends upon the shoot for sugar, thiamin, and vitamin B<sub>6</sub>, and further research may add to the growth substances concerned in the development of the tomato root.

#### SUMMARY

Light brown sugar was found to be more beneficial for the growth of excised tomato roots than pure cane sugar in a solution containing sugar, minerals, and thiamin. Growth decreased as the proportion of light brown sugar in the medium was reduced. The ash of the brown sugar was not responsible for the benefit observed. Nicotinic acid, nicotinamide, and amino acids were not beneficial when added to a solution of pure sugar, minerals, and thiamin, but crystalline vitamin  $B_6$  was quite beneficial. Vitamin  $B_6$  increased growth when added to solutions of sugar, minerals, and thiamin in which the proportion of brown sugar was reduced. Observations are reported on hooks and curls which developed in roots grown in solutions containing vitamin B<sub>6</sub>; these observations are interpreted to indicate the existence of a growth substance necessary for cell elongation in roots. Evidence for an unknown growth substance in samples of maltose is presented.

THE NEW YORK BOTANICAL GARDEN, AND DEPARTMENT OF BOTANY, COLUMBIA UNIVERSITY, NEW YORK CITY

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# PHYSIOLOGICAL RELATIONS BETWEEN HOST AND PARASITE IN CROWN GALL—AN EXAMPLE OF BASIC BIOLOGICAL RESEARCH WITH PLANT MATERIALS <sup>1</sup>

### A. J. Riker

The botanist who undertakes studies of the cell both in health and disease has certain important opportunities. It seems fitting on this centenary of the cell theory that emphasis should be placed both on the importance of cellular pathology and on certain advantages plant materials have over animal materials for basic research in this field. Some of these advantages may be briefly listed as follows:

(1) large numbers, (2) low cost, (3) ease of experimental use, (4) satisfactory physiological variability, (5) easily induced epidemics, and (6) genetic purity of hosts through (a) selfing and (b) vegetative propagation. The last is particularly important since pathogenicity must be defined in terms of resistance or susceptibility of the host.

As an example of the opportunities offered by plant materials, I am to discuss certain points in research on atypical and pathological multiplication of cells. These investigations center about the well-known plant disease, crown gall, which is caused by the bacterium, *Phytomonas tumefaciens* (Smith and Town.) Bergey et al.

Our primary interest in this topic is to determine the means whereby the bacteria, within the host tissue, are able to stimulate the great increase both in cell size and number—i.e., hypertrophy and hyperplasia. Thus, we are in a measure continuing the work so ably carried on for a number of years by Erwin F. Smith. You will remember that he pointed out various points of analogy between this plant disease and a very important hu-

<sup>1</sup> Received for publication February 1, 1939. This paper in somewhat extended form was given December 28, 1938, at Richmond, Virginia, before Section G, A. A. A. S., in a joint session between various affiliated

Botanical Societies.

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man disease. However, my colleagues and I, both in our discussions and publications, are careful to avoid any such terms borrowed from medicine lest confusion arise over terminology and obscure the main point of our researches—i.e., the cause of pathological growth.

The parasitic nature of this disease does not seem to separate this research from that in which no microorganism has been found associated with pathological growth. Such growth is easily induced at will in plants by a number of physical and chemical agencies. From the standpoint of the crown gall bacteria, and various other cell stimulating microorganisms, the atypical growth induced is apparently the result of physical or chemical changes occurring in the host tissue. An analytical study of what these changes may be places work involving microorganisms on much the same basis as work where no pathogens are known. However, with microorganisms as research tools, it is possible to study their metabolism of known materials in vitro as well as in vivo. With them one can induce in the localized tissues many small but continuously operating changes which seem more difficult with non-living agencies.

Only a few of the high spots, more particularly in relation to our own work,<sup>2</sup> can be mentioned this afternoon. Detailed references to the literature are

<sup>2</sup> The research at Wisconsin is supported jointly by the International Cancer Research Foundation, the University of Wisconsin, and the Wisconsin Alumni Research Foundation. It is carried on through cooperation between Professors I. L. Baldwin, Bacteriology, B. M. Duggar, Plant Physiology, W. H. Peterson, Biochemistry, and A. J. Riker, Plant Pathology, and their various associates whose names appear on the detailed publications. Aid has been received from the University of Wisconsin W.P.A. Natural Science Research Project.

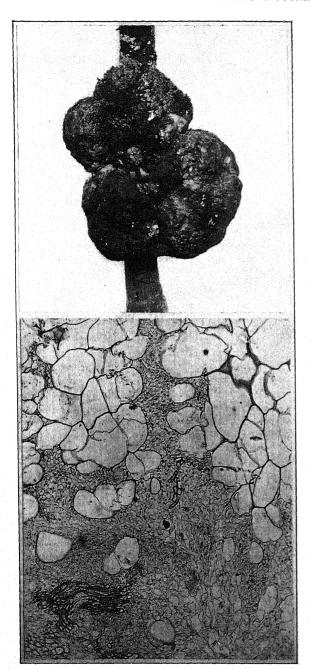


Fig. 1 (above). Crown gall on apple twelve weeks after inoculation with *Phytomonas tumefaciens* on the scion under ground.

Fig. 2 (below). Photomicrograph of crown gall on tomato. Hypertrophic, hyperplastic, and unorganized vascular tissue are evident.

omitted because of their large number, and because most of them are readily available elsewhere.<sup>3</sup> You will doubtless be interested to know that an exten-

<sup>3</sup> For example: Levine, M. 1936. Plant tumors and their relation to cancer. Bot. Rev. 2: 439-455; Riker, A. J., and T. O. Berge. 1935. Atypical and pathological multiplication of cells approached through studies on

sion of this subject is to be considered in a symposium being arranged by Dr. B. O. Dodge on "The effect of microorganisms, their products, and other chemical agents on the production of atypical growth in higher plants." It will be held next September in New York as a part of the program of the Third International Congress for Microbiologists.

The gross appearance of crown gall may be observed from figure 1. This type of growth is caused by an enormous development of cells, both in size and in number, which apparently lack organization.

A histological examination indicates that there is both hyperplasia and hypertrophy (fig. 2). Both the gross appearance and the anatomy of crown gall have many characters strikingly similar to those found in animal tumors or cancers. These characters are so well known that they are omitted in favor of some physiological considerations.

Our problem is perhaps most easily defined by considering a drawing made over a photomicrograph (fig. 3). Here we see crown gall bacteria (a) in an intercellular space of tomato pith eight days after inoculation. The stippled cells have laid down new walls (b) in the corners of the old pith cells. The nuclei (c) occupy a position close to bacteria. Although the bacteria have been seen

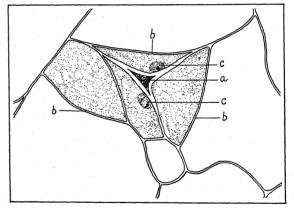


Fig. 3. Drawing made over a photomicrograph of tomato pith tissue eight days after crown gall inoculation. The bacteria (a) have reacted with the host tissue so that new walls have been formed (as at b) cutting off the stippled cells from the original pith cells. The nuclei (c) lie close to the position of the bacteria.

in the intercellular spaces and cultivated from them, this position may be of minor importance in a consideration of the physiological relation between host and parasite. The aim of our research is to determine the physiological interaction between bacteria and surrounding cells which starts pathological growth and which keeps it going.

crown gall. Amer. Jour. Cancer 25: 310-357. Abstracts of various recent contributions occur in the American Journal of Botany and Phytopathology.

This crown gall question may be approached from several directions. Just as Julius Caesar began his commentaries, "All Gaul is divided into three parts," we can divide our crown gall topic into three sections and consider in order (1) bacteriological, (2) biochemical, and (3) physiological aspects. Like many political divisions, our divisions here are rather arbitrarily made. I am particularly grateful for the opportunity to present before you this afternoon some ideas, perspectives, and results (some of which have been drawn freely from the literature) because I am confident of your help. Just as certain Romans stabbed Caesar for the good of "Democracy," I am sure you will puncture any false concepts and interpretations you detect for the good of this research program.

BACTERIOLOGY.—The pathogenicity of the crown gall bacteria in single cell culture has been examined in the hope that factors influencing its rise and fall might lead to an understanding of the means by which it stimulates cell growth. A variety of methods have been employed in an attempt to attenuate the crown gall bacteria. This was finally accomplished by carrying a culture through 10 to 20 successive transfers in a liquid medium containing glycine, a simple amino acid. Some related compounds have given similar results. While loss of pathogenicity appears in at least one case to have occurred spontaneously, it can be induced at will by this means. It also appears that the pathogenicity of glycine attenuated cultures may be gradually regained when they are restored to stock media. Frequent use of single cell cultures, at the beginning and at various intermediate points in these different steps, has satisfied us that the change is actually one in the organism itself, and quite independent of any question of mixture in the cultures. Whether one wishes to call this an induced variation, a mutation, or merely "education," is beyond the scope of this discussion. The essential fact is that pathogenicity can be increased and decreased at will by influencing the bacterial metabolism with certain of the simpler organic nitrogen compounds.

The substrata which these bacteria are able to utilize have been examined to determine what materials could be employed. Information is available concerning the utilization of all the common sources of carbon and nitrogen. We have often heard that Caesar crossed the Rubicon but kept his commentaries dry. So to avoid excessive desiccation, I shall pass over the details of these studies except to indicate that the bacteria will use most of the sources of carbon or nitrogen which they find in the plant, and many others besides. Likewise, as mentioned later, various chemical analyses of tomato stem tissue and of other host plants have shown the presence of a number of available nutrients, from some of which cell stimulating factors might be produced.

The changes the crown gall bacteria bring about in the host tissue can be roughly approached by studies with known materials in vitro.

The physical chemical changes investigated include (1) osmotic pressure, (2) hydrogen-ion concentration, (3) oxidation reduction potential, and (4) viscosity. In all but the last case, attenuated and pathogenic bacteria induce apparently similar changes. The pathogenic cultures have lower viscosity than attenuated cultures. However, this may be associated with differences in gum production and rate of growth.

BIOCHEMISTRY.—The products in the culture media, aside from the bacterial cells, depend in large part on the materials used and the conditions of incubation. From sugar, a considerable quantity of CO<sub>2</sub> and bacterial gum is produced. Studies of carbon balance show that there remains considerable unknown material, probably related to gum. When sugar was present, it had a sparing action on common organic sources of nitrogen. From peptone and its derivatives this organism, like many others, produces various materials among which ammonia and beta-indole-acetic acid have been identified. Here again attenuated and pathogenic cultures appear similar. In a synthetic medium both also produce vitamine B<sub>1</sub> in approximately the same high quantity as yeast.

In connection with the closely related hairy root bacteria it is interesting to note that some acidity is produced from sugar. The acids have been identified as acetic and pyruvic. The latter was recognized some time ago as capable of inducing root formation from certain plant tissues.

From the standpoint of the host plant, it appears that crown gall tissue is somewhat similar to embryonic plant tissue not only in certain histological characters but also in gross chemical composition. In the host there are obviously abundant sources of carbon and nitrogen in forms available to the bacteria. However, in comparison with uninfected stem tissue the gall tissue is particularly rich in oxidizing enzymes, such as catalase, oxidase, and peroxidase. The difference in tyrosinase content is still more striking. QO<sub>2</sub> measurements show, when comparisons are made on the basis of total nitrogen content, that crown gall tissue respires much more rapidly than corresponding stem tissue.

The hydrogen-ion concentration of tomato crown gall and contiguous uninfected stem tissue seems not to be significantly different, although variations in the two types of tissue have been found with other host plants. However, when a platinum electrode was inserted in gall tissue there was consistently a more positive charge than when such an electrode was placed in nearby stem tissue. This is in contrast to the fact that the bacteria lower the oxidation-reduction potential of the medium in which they grow.

PLANT PHYSIOLOGY.—Galls similar in general appearance to crown gall have been induced by various workers with a long list of different chemicals, and certain physical agencies. Some non-parasitic galls, which have similar histology as well as gross appearance, may be induced on certain plants by

successive treatments with strong (e.g., 3 per cent) beta-indole-acetic acid.

This or some other growth substance seems clearly to be associated with crown gall development as shown by (1) epinasty, (2) suppression of axillary buds, (3) secondary thickening of stems, (4) suppression of abscission layer, and (5) root stimulation. There is some evidence that the difference between the pathogenic crown gall culture and the attenuated culture may be something of this character. When the attenuated culture alone is punctured into a decapitated tomato stem there is little response of the host tissue. However, when it is below a crown gall, it produces galls practically as large as the virulent culture. With certain other conditions an attenuated culture induces adventitious shoots on tomato.

The amount of growth substance in culture or in crown gall tissue is apparently small. The largest amount found in a crown gall culture grown in peptone glucose broth was equivalent to 120 gamma beta-indole-acetic acid per liter. The amount found in certain gall tissue varied with circumstances in this general range. This is to be compared with 3 per cent (30,000,000 gamma per liter) beta-indole-acetic acid used directly to stimulate growth. The material found in host tissue seems more sensitive to acids than to bases, which suggests that it is related to beta-indole-acetic acid.

The "host ranges" of crown gall and of betaindole-acetic acid are not parallel, crown gall occurring readily on various plants where this acid is not effective in gall production. It would be surprising if various precursors and accessory factors were not involved. These and several other lines of evidence suggest that judgment should be reserved about beta-indole-acetic acid being the primary cause for the pathogenicity of crown gall bacteria.

The increase in chromosome number of crown gall cells has been reported by several workers. The chromosomes in legume root nodules are doubled. The studies just discussed by Dr. Blakeslee on increasing chromosome numbers with chemicals raise the question whether metabolites from crown gall bacteria may cause similar responses.

Some discussion seems desirable of the meaning of these various results. The crown gall literature, which borrows many of its concepts from medicine, contains a large number of working hypotheses aimed at explaining the cause of this pathological growth. These hypotheses are too numerous for discussion here. Perhaps the present most popular concept extends an older idea-viz., that the host and bacteria interact and produce a cell stimulating chemical which starts the cells on their diseased multiplication. However, it seems unwise to overlook other possibilities. For example, the physical chemical changes involved have many promising aspects. Likewise, instead of a cell stimulating material being produced, a cell regulating or inhibiting substance may be removed. The factors which start off the gall may be different from those which keep it going. From the foregoing discussion of results, I hope it is apparent that a large number of working hypotheses have been tested and found wanting. One of the greatest handicaps in this general type of work has been the lack of adequate controls on the experiments performed. Fortunately for the crown gall investigations, such checks have been developed.

Two important safeguards are worth listing. (1) In relation to the bacteria, attenuated cultures can be carried in parallel with pathogenic cultures. At least quantitative differences should be found before attributing a rôle in pathogenicity to any factor tested. (2) In relation to the tomato host, treated plants can be maintained below 28°C. where galls develop and above 30°C. where they do not. It appears that any factor responsible for pathogenicity should be much less active if not absent at the higher temperature. By means of these and similar controls research workers may avoid premature conclusions.

This work on cell stimulation with crown gall serves, I hope, as an illustration of the opportunity for attacking various basic problems in biology with plant materials.

Our colleagues, who work with animal materials, may consider that I am a "radical red" for placing so much emphasis on some of the superior advantages of using plant materials. If I should appear to be reflecting long wave lengths of light, I protest it is not because of radical tendencies, and not even of Christmas celebrations, but merely because of a glow of enthusiasm over the opportunity provided by plants for studying basic biological problems.

DEPARTMENT OF PLANT PATHOLOGY, UNIVERSITY OF WISCONSIN, MADISON, WISCONSIN

# THE PRESENT AND POTENTIAL SERVICE OF CHEMISTRY TO PLANT BREEDING 1

Albert F. Blakeslee

Your secretary, in asking me to present a paper before Section G at this meeting, suggested I report on our recent investigations with colchicine. I shall speak about colchicine but shall use this drug chiefly as an example of how chemical substances may be used in control of life processes. Like all findings, the use of colchicine to influence chromosome behavior has a history. Dustin<sup>13</sup> of Brussels first reported its action in arresting nuclear divisions. Allen introduced it to endocrinologists in this country to determine relative abundance of nuclear divisions in animal tissue. Eigsti,14 Nebel,18 and perhaps others as well as ourselves independently applied the drug to the induction of extra chromosomal types in plants. The successful use of colchicine for this purpose happened to fit into our research program.

COLCHICINE IN THE CORM OF COLCHICUM.—Colchicine occurs in the seed and corm of Colchicum autumnale (meadow saffron). The corm may contain as much as 0.4 per cent by dry weight. A concentration of 0.4 per cent in water solutions will induce doubling of chromosome number in Datura, and one-thousandth of this concentration will induce chromosome doubling and distinct swelling in seedlings of Portulaca. The Colchicum plant is not affected by the colchicine in its own cells nor is there any swelling of roots or doubling of chromosomes evident when the roots are immersed in strong concentrations of the alkaloid colchicine. It is like the snake which is immune to its own venom. We have been unsuccessful in attempts to extract an "anticolchicine" from the corm which would inhibit the action of colchicine on chromosomes of other plants. Perhaps someone will suggest how we can get out such an anticolchicine, provided the immunity of the chromosomes of this plant to its own alkaloid colchicine is not perhaps inseparably tied up in some way in the nature of the species protoplasm. We should, however, prefer to have it due to an extractable chemical compound, which we would find useful in our experiments. A few other chemicals have been found by us to have an effect similar to that exercised by colchicine, but none so far has been found which is as effective in inducing chromosome doubling. By the use of sodium cacodylate we have induced plants with doubled chromosome number in Portulaca. Acenaphthene, which Kostoff<sup>17</sup> reports having used to double chromosome numbers, seems relatively ineffective in inducing 4n plants in the species with which we have tested it.

<sup>1</sup> Received for publication January 27, 1939.

Invitation paper presented before the joint session of Section G of the A. A. A. S., the Botanical Society of America, American Phytopathological Society, American Society of Plant Physiologists, Mycological Society of America, Sullivant Moss Society, and American Fern Society, December 28, 1938.

PLANTS TREATED WITH COLCHICINE.—As is shown by table 1, in little over a year by the use of colchicine we have succeeded in doubling the chromosome number of 65 different kinds of plants if we include varieties as well as species. These are included in 41 different species, 24 genera and 14 families of flowering plants. Some in the table have been already reported by Avery and the

## CHROMOSOMES DOUBLED BY COLCHICINE

```
CARYOPHYLLACEAE
                                                                                    MORACEAE
       LYCHNIS DIOICA **
STELLARIA MEDIA **
VACCARIA PARVIFLORA **
                                                                                           CANNABIS SATIVA **
                                                                                    OXALIDACEAE
 CHENOPODIACEAE
                                                                                          OXALIS VALDIVIENSIS **
                                                                                    PLANTAGINACEAE
 COMPOSITAE
                                                                                           PLANTAGO LANCEOLATA
      BIDENS LEUCANTHA 女会
COSMOS SULPHUREUS 女女
RUDBECKIA HIRTA 女
                                                                                    POLEMONIACEAE
                                                                                           COLLOMIA COCGINEA A
 CRUCIFERAF
                                                                                    PORTULAÇAÇEAE
       RAPHANUS SATIVA
                                                                                          PORTULACA GRANDIFLORA PORTULACA MARGINATA PORTULACA PORTULACA PARANA PARANA
 CUCURBITACEAE
     UCURBITACEAE

CUCURBITA PEPO
MAMMOTH PUMPKIN
PEAR GOURD
SMALL ROUND CHIMA GOURD *
"SPOON" GOURD *
WHITE SPHERE SOUASH *
YELLOW DISK SOUASH *
YELLOW DISK SOUASH *
CHURCH SOUASH *
CHURCH SOUASH *
CHURCH SOUASH *
CHURCH SOUASH *
CUCURBITA MOSCHATA
SMALL YELLOW GUSHAW SOUASH *
CUCURBITA MAXIMA X C. MOSCHATA *
LAGENARIA VULGARIS
DIPPER GOURD *
GIANT BOTTLE GOURD
KNOBKERRE GOURD
HERCULES CLUB GOURD
HERCULES CLUB GOURD
                                                                                    SOLANACEAE
                                                                                          DATURA CERATOCAULA XX
DATURA DISCOLOR X
DATURA FEROX XX
DATURA INNOXIA X
DATURA LEICHHAROTII XX
DATURA METEL XX
DATURA METELOPES
DATURA METELOPES
DATURA METELOPES
                                                                                          DATURA QUERCIFOLIA *
DATURA STRAMONIUM *

6 MAIN LINES AND GENE TYPES *

8 RACES WITH EXTRA CHROMOSOM
                                                                                        MATERIAL &
NICOTIANA TABACUN X N. GLUTINOSA X X
NICOTIANA TABACUN X N. GLUTINOSA X X
NICOTIANA TABACUN X N. SLUVESTRIS X X
PETUNIA AXILLARIS X X
EUPHORBIACEAE
       MERCURIALIS ANNUA
                                                                                    VIOLACEAE
MALVACEAE
                                                                                          VIOLA TRICOLOR, HORTENSIS
       ANODA LAVATEROIDES
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Table 1. List of plants with chromosomes doubled by colchicine.

# 4H SEED # = 4N OFFSPRING

speaker in a recent issue of the Journal of Heredity. The chromosomes of the others we have doubled with the help of Warmke. The list would be considerably larger if we included examples from other investigators. It is becoming apparent that the use of colchicine among the flowering plants to double the chromosome number is of rather general application, although there is considerable difference between even related species in respect to susceptibility to treatments. In addition to the species listed in the table, Miss Satina has doubled the chromosome number in Marchantia polymorpha, and we now have 2n male and female gametophytes by treating gemmae of this liverwort. Preliminary tests of some algae indicate that they also may be susceptible; at least colchicine is toxic to them and induces swollen cells. The fungi seem immune. Miss Satina has tested representatives from a wide range of families of fungi. She finds that in aqueous solutions or in agar media containing from 0.4 to 4 per cent colchicine, the fungi grow as well as in the controls. Others have told us of similar experience with fungi.

Characteristics of tetraploids.—With the accumulation of types with doubled chromosome number from an increasing number of groups, it will be possible to speak with more confidence regarding the effects which are brought about by the increase in chromosome material. We can touch upon only some of the more obvious morphological effects. The more important physiological effects must be left for further study. In discussing the appearance of tetraploids, a distinction must be

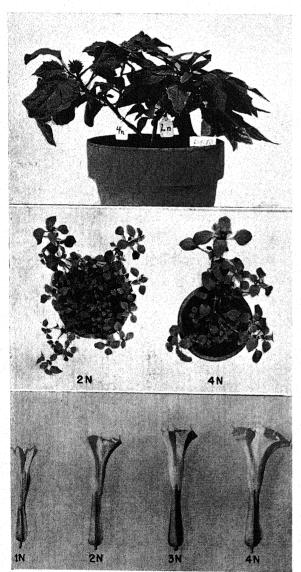


Fig. 1-3.—Fig. 1 (above). Normal 2n plant of Datura stramonium with 4n mutant branch on left.—Fig. 2 (center). Chickweed (Stellaria media). 2n plant on left, 4n on right.—Fig. 3 (below). Flowers of balanced types of Datura stramonium. From left, 1n, 2n, 3n, 4n.

made between the condition in the plant in which the tetraploidy first appears and the condition in an established 4n race. Figure 1 shows a potted plant which was photographed in 1921. The right side of the plant is normal 2n, the left side with broader roughened leaves and depressed capsule is a 4n mutant branch. The fact that spontaneous tetraploids (4n) almost invariably appeared as sectorial chimeras with roughened leaves containing mixed 2n and 4n tissue was one reason for our belief that spontaneous doubling of chromosomes in Datura at least must be brought about in somatic tissue rather than in the germ cells and must be susceptible to environmental influence. Nemec in 1904<sup>19</sup> obtained cells with doubled number of chromosomes in roots by treatment with chloral hydrate. Our earlier experiments with chloral hydrate, however, and with high and low temperatures were not successful in inducing 4n individuals. Figure 1 might serve as an illustration of a plant which had grown from a seed treated with colchicine, since it shows both the 2n and 4n branches which may develop from such treated seeds. The roughening of the leaves is also characteristic of the early effects of colchicine treatment. Ultimately smooth-leaved branches may grow out of the rough-leaved tissue. and in the next generation clean tetraploids (4n) may be expected. There is some evidence that colchicine-treated plants in the first generation may be periclinal chimeras, with the outside of the plant having a different number of chromosomes from the inside. If this turns out to be the case, we may have a method of determining definitely, for Datura at least, what layer, or layers, in the stem gives rise to egg cells and pollen grains. In view of the possibility of periclinal as well as sectorial chimeras, it has been our practice to establish tetraploid races by planting seeds from induced 4n branches before attempting critical studies on the differences between tetraploids and diploids. Tetraploids are characterized by a general greater robustness in comparison with diploids. The common chickweed (Stellaria media), in which we doubled the chromosomes unintentionally by accidental spraying with colchicine solutions, is shown in figure 2 with a normal 2n plant on left and a 4n plant on right. The leaves are characteristically broader, and stems and leaf stalks are thicker. It seems to be a general rule that leaves of tetraploids are darker green than those of diploids and a 4n sector on a 2n stem in some cases may be recognized by its darker green color. We have observed a deeper color in the flowers of tetraploid yellow cosmos which may also be due to a thicker layer of colored plastids.

As the balanced number of chromosomes increases in Datura from 1n through 2n and 3n to 4n, the flowers become progressively larger, as shown in figure 3. We have obtained 8n flowers by colchicine treatment, but such flowers have so far failed to set seed. By treating buds of 3n plants, we have obtained 6n flowers and capsules, but they have given us only very few seeds, and these have failed to

germinate. There seems to be a limit to the number of times the chromosomes of a species may be doubled and give rise to polyploid races of higher order. That increased size of flowers goes with doubled number of chromosomes seems to be a general rule. The flowers in figure 4 are typical representatives from individual offspring of a single 2n plant of Petunia, one branch of which had been treated with colchicine and induced to form 4n tis-The flowers on the left came from 12 offspring from an untreated 2n branch and are diploid. those on the right came from 12 offspring from the 4n branch and are tetraploid. Although genetic and environmental factors have an influence on the size of flowers in this species, it is obvious that doubling the number of chromosomes is of primary importance in causing the 4n flowers to average considerably larger than the 2n flowers. In certain other species such as Portulaca, however, environmental factors appear of more influence on the size of the flowers than doubling the number of chromosomes. In each pair of plants in figure 5 the

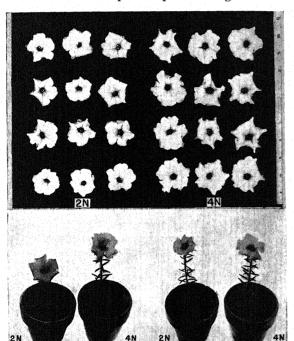


Fig. 4 (above). Flowers of garden Petunia; the 12 on left 2n, the 12 on right 4n.—Fig. 5 (below). Portulaca parana. In each pair, plant on left is 2n, that on right 4n.

plant on the left is 2n and that on the right 4n. In the right pair the flower of the 4n plant seems larger than that of the 2n, but this relation does not hold in the left pair. Neither in this species (P. parana) nor in the more common species, P. grandiflora, could one readily distinguish a 4n from a 2n plant by the size of the flowers alone. The greater thickness of the 4n leaves would be a better criterion.

In Datura the capsule tends to become progressively shorter and thicker as we go from the 1n

through the 2n and the 3n to the 4n types.<sup>4</sup> The shape in this series, however, is somewhat affected by a paucity of seeds in the uneven balanced 1n and 3n capsules. In general, doubling chromosome

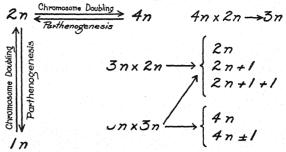


Fig. 6. Diagram showing origin from normal 2n of 1n, 4n, and 3n balanced types; and from 3n origin of 2n + 1 and 2n + 1 + 1 unbalanced types.

number makes the fruits stouter, but the effect is not uniform in all forms. An interesting series of changes in size and shape of fruit following doubling of chromosome number is shown in the Cucurbitaceae. Dr. Sinnott,<sup>23</sup> with whom we have been cooperating in a study of the effects of extra chromosomes upon morphological and anatomical structure, is attempting to relate the different types of changes in the fruits to differences in their constituent cells in respect to their number, size, and planes of division.

TETRAPLOIDS THE SOURCE OF OTHER CHROMO-SOMAL TYPES.—Doubling chromosome number not only forms a new type of plant, but, as we have shown for Datura, it also furnishes a starting point for a considerable series of new types. (The last cited paper gives a 19-year bibliography of our Datura investigations.) As shown in figure 6, we may start with a 4n individual and by crossing it

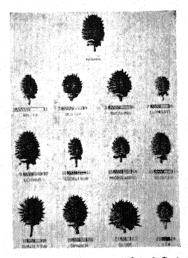


Fig. 7. Top, a normal 2n capsule of  $Datura\ stramonium$ ; below, capsules of the 12 primary 2n+1 types arranged from upper left to lower right in order of decreasing size of the chromosome which is extra.

with a normal 2n get a 3n plant. The 3n may then be selfed or, better, crossed with a 2n to give the full range of primary simple trisomic (2n+1) types each with a different extra chromosome (fig. 7). In addition, double trisomic (2n+1+1) types are to be expected in relatively large numbers. Such trisomic types have been of value in analyzing the content of genes in the individual chromosomes by the changes brought about when they are present as extras and have been used in  $Datura^{11}$  and maize<sup>20</sup> in locating genes by trisomic ratios.

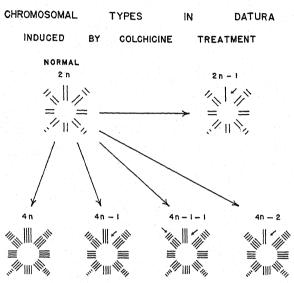


Fig. 8. Chromosomal types in Datura induced by colchicine treatment.

CHROMOSOMAL DEFICIENCIES FROM COLCHICINE TREATMENT.—We have spoken as if doubling chromosome number were the only change induced by colchicine. That this is not the case we were early led to conclude from the appearance of plants grown from treated seeds. This last summer we had growing in the field more than two thousand Datura plants from seeds which had been treated with 0.2 and 0.4 per cent solutions of colchicine for different lengths of time from 3 to 8 days. From all these treatments, plants with abnormal sectors were abundant. They amounted to from 55 to 77 per cent from the 0.4 per cent treatments. While most of the abnormal sectors could be classified as  $\pm 4n$  by appearance, many were obviously modified 2n or 4n branches. With the help of Avery we selected a couple of hundred for cytological study by Dr. Bergner. Figure 8 shows some of the simpler chromosomal types that were identified from study of pollen mother cells. Of the 2n-1type at the right, we have identified 7 cases. One of these had lost the largest (1.2) chromosome as indicated in the diagram. We have already spoken of the value of the 12 primary 2n+1 types in Datura. Capsules of these are shown in figure 7. By the use of colchicine we now have a method of getting 2n-1 types and should be able to learn more

about the factors in individual chromosomes by comparing the effects brought about by the loss of a given chromosome with those brought about by the presence of the same chromosome as an extra. It may be difficult to secure the full twelve 2n-1 types, however, and when found, they will have to kept going by grafts and cuttings, since they cannot be propagated by seed, because deficiencies are not viable in the gametophyte. On the lower line of the diagram are shown 4n and modified 4n types. Of these the simplest was brought about by the loss of a single chromosome after doubling had taken place to give 4n-1 types. There were 16 4n-1 branches identified. Of those which had lost two chromosomes there were two types. In the first, a single chromosome had been eliminated from each of two sets to form 4n-1-1types. In the second, two chromosomes were lacking from a single set. Such 4n-2 types could have arisen from doubling the chromosomes of a 2n-1 type after the elimination of its deficient chromo-

There were 10 of the 4n-2 types to 2 of the 4n-1-1 types. The 4n-2 types may be expected to form 2n-1 gametes and to breed true for the deficiency. They should give disomic inheritance for genes in the set with the deficiency and tetrasomic inheritance<sup>9</sup> in other sets. If their reduced eggs could be induced to develop parthenogenetically, we should have a further source of 2n-1 plants. Another source would be the 4n-1 types, half of the eggs of which should be 2n-1. Various combinations of the modified 4n types given in the table have been identified. The most complicated had 42 chromosomes with a chromosomal constitution represented by the formula 4n-1-1-1-2.

Doubling chromosomes in haploids.—We have spoken of doubling chromosome numbers of diploids. They may be readily doubled also in haploids. Haploids of some species never produce seeds, and in some races of *Datura stramonium* our efforts to secure seeds have been fruitless. In our main line an occasional capsule on older haploids



Fig. 9. A haploid (1n) plant of D. stramonium which has been sprayed with colchicine. Note the 2 large 2n capsules toward right and left of picture which had been induced by the treatment. A small typical 1n capsule is seen at upper center.

will be found to have formed one or two seeds by non-reduction, but the large majority of haploid flowers are sterile. In figure 9 we have a photograph of a haploid plant which has been sprayed with a 0.4 per cent solution of colchicine. The two large diploid capsules which have been induced by the treatment are in contrast to the typically small 1n capsule at the upper middle of the picture. The capsule of intermediate size half way to the left is perhaps a mixture of 1n and 2n tissue.

Now that we have learned how to induce haploids to double their chromosome number and become diploids, how can we use this knowledge in a plantbreeding program? We could make great use of it if we could only induce the production of haploids. As shown by figure 10, we could start with a highly heterozygous plant such as a fertile species hybrid and by parthenogenesis of a reduced egg obtain a haploid with only one of each kind of chromosome. Each of these haploid chromosomes could be doubled by treatment with colchicine to give homozygous seeds with each kind of chromosome duplicated. All we need to get pure lines from heterozygous material by two jumps is a method of inducing parthenogenesis. Since we first identified a haploid in 1921 we have recorded the spontaneous appearance of 216 haploids in Datura, 22 but we do not yet know the factors responsible for their occurrence.

# 2n HETEROZYGOUS

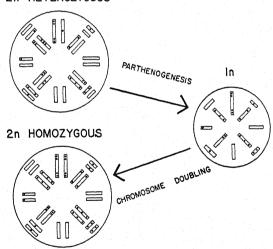


Fig. 10. Diagram showing method of securing pure lines from heterozygous material.

Last winter Warmke found that by treating unpollinated pistils with indol-butyric acid, he could induce them to develop into small capsules, which, however, never formed seeds. Buchholz had earlier noticed that he could induce formation of seedless capsules by applications of pollen which had been treated with heavy dosages of X-rays. This last summer Warmke and Buchholz combined the two methods but without success in inducing parthenogenetic seeds. Other stimuli are being tested with both 2n and with 4n parents. Success seems more

likely with the latter, since 2n in addition to 3n offspring are occasionally induced by the cross  $4n \times 2n$ . We should be glad of any suggestions how to induce parthenogenesis since this is one of the things we are eagerly yearning to do.

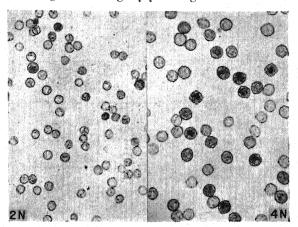


Fig. 11. Pollen of hemp, from 2n flowers on left, from 4n flowers on right. Note larger grains in latter.

Polyploidy and sex.—Among the problems connected with polyploidy is the effect of doubling chromosome number upon the sex of dioecious plants, which is being studied with Warmke. Hagerup's discovery in the arctic regions of a tetraploid hermophroditic species, Empetrum hermaphroditum, 16 which is closely related to the diploid dioecious species E. nigrum gave presumption for the belief that doubling chromosome number would change a dioecious into an hermaphroditic species. Experiments with hemp and Lychnis show that tetraploids, at least of these species, remain dioecious. Among tetraploids of spinach we have gotten male, female, and hermaphroditic plants.

Some of the difficulties of working with dioecious forms may be illustrated by our experience with hemp. Although organs of a 4n branch are more robust than those of a 2n branch, more reliable criteria than general appearance are needed, espe-

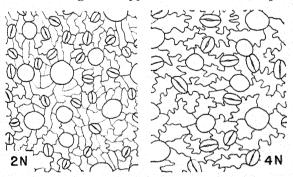


Fig. 12. Drawings from surface of 2n (left) and 4n (right) leaves of female hemp. Note larger size of epidermal and especially guard cells in 4n leaves. Circles represent cross sections of glandular hairs which are not greatly affected in size by chromosome number.

cially for the treated generation. The larger size of pollen grains from a 4n flower, shown in figure 11, is a good criterion for male plants but cannot be applied to females. The larger size of

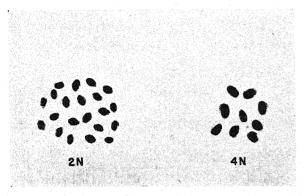


Fig. 13. Seeds from 2n (left) and 4n (right) flowers of hemp. Note larger size of latter.

stomata, shown in figure 12, is characteristic of tetraploids but holds strictly only for comparable leaves. In the figure it will be noted that the epidermal cells as well as the guard cells are larger in the 4n leaf but that there is no material difference in the size of cross sections of the glandular hairs which are shown by circles in the drawing. Stomatal size was found a reliable criterion when the stomata were taken from the floral bracts of females. In figure 13 are shown 2n and 4n seeds

of hemp. In all species so far examined, the 4n are distinctly larger than the 2n seeds.

MULTIPLE DIPLOIDS.—We are most of us familiar with sterile hybrids between plant species. They are like the sturdy mule in that they cannot form gametes since the chromosomes of one parent are too unlike those of the other parent to mate and form pairs; and pairing of chromosomes is necessary for sexual reproduction. Very rarely such sterile hybrids have been rendered fertile by spontaneous chromosome doubling. Now, however, we are able to induce such doubling at will by chemical How this is brought about may be treatment. clearer from the diagrams of figure 14. The chromosomes of species A are represented by solid lines, those of species B by dotted lines. Each species is assumed to have 4 different sets or kinds of chromosomes. In the sporophyte there are 2 chromosomes in each set, but in the gametophyte and gametes the number is reduced to half, and each set contains only one chromosome. In the hybrid between species A and B the chromosomes are assumed to be unable to pair, with resultant sterility of pollen and egg cells. In the sterile hybrid there is only one chromosome in each set but there are now 8 sets or as many sets as in both the two parents combined. Such a sterile hybrid, therefore, is a double haploid. By the use of colchicine the chromosomes may be doubled. The resulting type will be diploid, since it has two chromosomes in each of the 8 sets. It will be fertile, since each of the chromosomes of both species A and B will have duplicates from their own species with which to

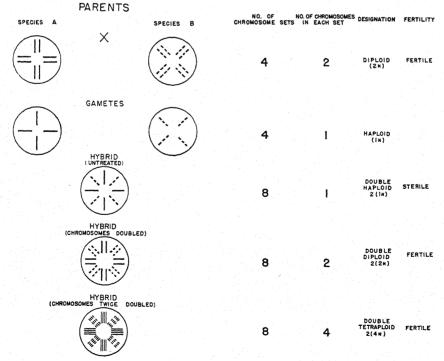


Fig. 14. Diagram to show method of induction of double diploids and double tetraploids from double haploids through doubling chromosome number.

pair. Since the number of sets is the sum of those from two parents, it may be called a double diploid. If the number of chromosomes were again doubled there would be 4 chromosomes in each set, and the plant should be called a double tetraploid.

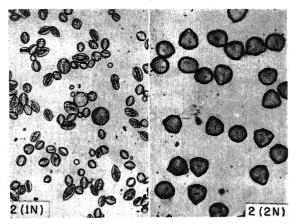


Fig. 15. Pollen from the hybrid *Nicotiana glutinosa*  $\times$  *N. sylvestris*. On left aborted grains from sterile double haploid flowers, on right good grains from fertile double diploid flowers.

The word tetraploid means 4-fold and would seem applicable only to cases in which each kind of chromosome is represented 4 times. The term amphidiploid as a Latin equivalent of our double diploid describes what actually happened to the chromosomes. Allotetraploid is an unfortunate term since it emphasizes the mere change in total number without regard to how this change has been brought

about. In a terminology regarding changes in chromosome number we believe it will give a clearer picture of what happens if we focus our attention upon the different kinds or sets of chromosomes the members of which are doubled, tripled, or quadrupled to form 2n, 3n, or 4n types. We suggest the terms simple and multiple polyploid instead of autoand allo-polyploid.

We have gotten the second generation of a double diploid race through the use of colchicine in doubling the chromosome number of a sterile hybrid between Nicotiana glutinosa and N. sylvestris,24 cuttings of which had been kindly sent us by Dr. F. O. Holmes. Sterile hybrids between these two species have been known for some time, but so far as we are aware, the fertile double diploid has not been reported before. From figure 15 it will be seen that the aborted pollen of the sterile hybrid can be readily distinguished from the good pollen of the induced fertile hybrid. In addition to the condition of the pollen, the setting of the capsules is good evidence that a double haploid has been transformed into a double diploid and become a fertile hybrid. Judging from the roughened leaves of a treated double diploid seedling secured from the cross N. tabacum  $\times$  N. glutinosa, 12 we are on the way to produce the double tetraploid shown in diagram in the lower part of figure 14.

In figure 16 is shown by diagrams the methods by which it might be possible to build up triple and quadruple diploids. If  $Nicotiana\ tabacum$  is already a double diploid, as specialists in this genus believe, 15 we have recently built up a triple diploid race from the sterile hybrid  $N.\ tabacum \times N.\ glutinosa$ . Cooperative studies on the genus Nico-

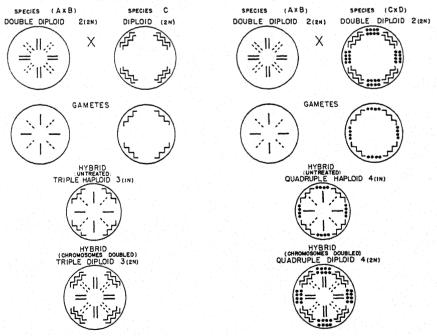


Fig. 16. Diagram to show method of induction of multiple diploids through doubling chromosome number.

tiana which are being undertaken with Dr. Holmes may give us before another Christmas meeting quadruple and even quintuple diploid races. A limitation to the formation of such multiple diploids is the number of hybrid combinations which are sterile because of chromosome incompatibility. In Datura, for example, multiple diploids have not been formed, since none of the hybrid combinations within the 10 herbaceous species are sterile from lack of chromosome compatibility. It seems unlikely that chemical methods could be found that would permanently prevent compatibility between chromosomes in these fertile hybrids, since this would appear to involve simultaneous mass mutations in all the chromosomes. It is more likely that genes might be found that would decrease compatibility between chromosomes belonging to different species but permit pairing of chromosomes belonging to the same species. This is suggested, since there are already known genes in Datura and maize which prevent pairing of chromosomes altogether within these species.

Multiple diploids are of interest to the student of evolution, since the evidence is strong that they represent a method of evolution of species in nature. We now have an opportunity to make new species to order.

Multiple diploids should have hybrid vigor like the mule, and the desirable characteristics brought about by doubling chromosome number. The possibilities in the way of new forms of economic value seem very great. In a number of cases sterile hybrids between species have been made fertile through spontaneous doubling of chromosome number which had taken place before recorded history. These fertile hybrid races were preserved by prehistoric man because of their superior qualities. Among such fertile races which have developed from species hybrids through doubling chromosome number may be mentioned our best varieties of wheat, oats, timothy, tobacco, and cotton. We now no longer have to wait ages for the chance hybridization between species and the later rare spontaneous doubling of their chromosomes in order to secure such superior varieties. We can now make them up to order.

We have told something about the recent work with colchicine as an example of how a chemical method can be of service to one branch of botanical science. In the few minutes remaining we shall suggest some other ways in which botany might be aided by chemistry.

IDENTIFICATION OF GENETIC TYPES BY CHEMICAL REAGENTS.—Many years ago we found a single plant of a yellow-coned variety of the Black Eyed Susan (Rudbeckia hirta) near the grounds of the Connecticut State College, and several similar yellow-coned plants some 7 or 8 miles away. Since Rudbeckia is self sterile, we crossed together the yellow-coned plant from the first locality with one from the second locality. To our surprise we got normal purple-coned offspring. The purple cones

in the F<sub>1</sub> and the type of segregation in later generations proved that we were dealing with two kinds of yellow cones, although we could not distinguish them apart by appearance. It occurred to us, however, that they must differ chemically and that it might be possible to tell them apart by use of some chemical reagents. We therefore tested the two kinds of yellow cones with all the substances we could find in the chemical laboratory. Since we knew no reason for selecting one reagent rather than another, we tried them out in the order in which the bottles stood on the shelves. We were rewarded by finding that strong KOH and NaOH (but not NH4OH) would turn the yellow cones of one race black and these we called the Black Yellow race, while the same reagents would turn the yellow cones of the other race red and these we called the Red Yellows. It was an easy task then for an assistant with a bottle of KOH to separate a segregating group of yellow-coned plants into the two genetic types, Black Yellows and Red Yellows.2 By a chemical method he could discover in one afternoon the genetic constitution of more yellow-coned plants than we could discover in a whole summer spent in making crosses to tester types and a second season in planting and recording the back-cross progenies. We believe that chemical methods of distinguishing other genetic types that look alike will ultimately be worked out. Especially useful to the breeder would be chemical methods of distinguishing homozygous individuals from those heterozygous for a given trait. Such methods would be most valuable with the slower breeding organ-The difficulty of distinguishing carriers of deleterious recessive traits from individuals free from them is the reason why geneticists doubt the possibility of eradicating or even greatly reducing the number of feeble-minded individuals, for example, in the human population by preventing the breeding of the homozygous recessives which alone can be recognized. A chemical method of recognizing the heterozygous carriers, if utilized, should enable the recessive trait to be eliminated from the population in a single generation. The reference to humans may indicate the tremendous importance which such chemical methods might have, but, as in many other biological problems affecting human beings, we shall probably have to look to botanists to lead the way.

OTHER CHEMICAL SERVICE NEEDED.—I shall merely mention some of the other ways in which I hope chemistry will be of service to plant breeding.

I want to have a method of inducing shoots to grow from any part of the plant—stem, root, or leaf. That this may be possible is suggested by the fact that certain species have this power to produce shoots to a marked degree. In Datura, moreover, the 17-18 chromosome when present as an extra induces abundant formation of adventitious leaves and shoots in continuous lines along the stem. The factors in this stimulus we have located in the 18

half chromosome. I had down in my notes to say that since Robbins and White had worked out the methods of growing excised roots in artificial media, we ought to be able to devise methods of growing tissue cultures of plants and be in a position to regenerate a whole plant from any single cell. I notice from the abstracts of this meeting that White has already started growing tissue cultures of callus cells.

We very much need chemical stimuli that will induce crossability or remove the block to crossability between species which we are attempting to investigate genetically.

We need also methods to induce a continuation of growth after fertilization in certain wide crosses. Considerable progress has been made by different investigators in getting arrested embryos to develop through to seedlings, but we are still looking for a method of coaxing the proembryo from the cross  $Datura\ stramonium\ imes\ D.\ metel$  to develop any further than an 8 cell stage. 21

We do not know what the chemical differences are in the environment within the ovules and anthers which bring about the reduction divisions of chromosomes. If we did know, we might be able to get reduction divisions in roots or in any other part that was most convenient.

We have frequently asked the *Drosophila* workers what makes the chromosomes behave the way they do in the salivary glands of their domesticated diptera so that they are 150 to 200 times their size in other parts of these insects. They can't tell us. If they could, we might be able to borrow some of

the chemicals which *Drosophila* uses and get giant chromosomes in some useful plant like the Jimson weed.

I am still enough of a mycologist to want to see some method developed of growing fungous parasites such as the rusts on artificial media. By rather simple adjustment to the nutritional requirements, Miss Satina and I have been able to grow the parasites Chaetocladium and Parasitella without the presence of the usual mucor hosts. With increasing knowledge of growth substances it ought not to be long before rusts are grown on agar in test tubes.

LEARNING BY YEARNING.—I have mentioned only some of the things I yearn to be able to do. Perhaps these may be considered botanical luxuries. It must not be forgotten, however, that civilization has advanced more by yearning for luxuries than by struggling for existence. What we need is to get more people yearning for things they don't know they want. So far as the probable results are concerned, I believe it would be more profitable to cut down on the lengths of papers at science meetings and to devote the time thus saved to periods of yearning-yearning by each for the luxuries that seem unattainable in his own scientific hobby. I find I have saved one minute and a half of the time allotted me and this I am donating to the section toward such a period of yearning.

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# THE RELATION BETWEEN STRUCTURE AND FUNCTION OF THE PHLOEM <sup>1</sup>

## A. S. Crafts

NEARLY THREE HUNDRED years have passed since Malpighi ringed plants to study conduction, and more than two hundred since Hales' memorable experiments on sap movement. Eighty years ago Theodor Hartig (1858) postulated that the principal movement of organic solutes occurs in the phloem. Only within the last decade, after years of devious experimentation, have plant physiologists returned to this view. Ringing experiments, chemical analyses of tissues and exudates, and studies with dyes, viruses, and radioactive elements support the view that rapid longitudinal transport occurs in the sieve tubes.

On the mechanism of movement in the sieve tubes, however, there is still controversy. As recently proposed (Crafts, 1938), workers in this field may be grouped as follows: (1) those who attribute rapid diffusional movement of solute molecules to a specialized activity of the sieve tube protoplasm and (2) those who picture a flow of solution through perforate or permeable elements resulting from a gradient of hydrostatic pressure developed osmotically. Views of the first have been termed the "protoplasmic" theories; those of the second, the "pressure flow" theories (Crafts, 1938). Huber (1937) has classified them as the "diffusion" and the "convection" theories.

Recent calculations indicate that acceleration of solute movement via the protoplasm must be several million times the normal rate of diffusion when it is based upon the actual space available within the protoplasm (Crafts, 1938). Advocates of the protoplasmic theories assume that this acceleration is accomplished by rapid streaming, activated diffusion, or the application of surface forces within the sieve tubes. Postulating the independent movement of different molecules, these theories demand the utilization of high energies (Mason and

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Phillis, 1937) to overcome the internal resistances of the system. In the pressure flow mechanism, solvent and solute are assumed to flow together as a solution through elements of specialized structure or permeability, the protoplasm of which plays an entirely passive rôle in the process. The sources of energy in this mechanism lie in the osmotic activity of the products of assimilation in green portions of the plant and in the accumulative ability of cells in growing and storing tissues.

With such widely differing views on the rôle of the sieve tube protoplasm in conduction, studies on the structure and vital activities of sieve tube elements should provide useful data for the solution of this problem.

PLASMODESMATA.—The classical concept of the sieve tube as a series of elements with end walls perforated to facilitate rapid longitudinal movement was developed largely from the works of Hartig (1854, 1858), Nägeli (1861), Strasburger (1891), and Hill (1908) and resulted from an attempt to reconcile microscopic impressions of sectioned tissues of lianas and woody angiosperms with the commonly observed phloem exudation. Though the earlier workers pictured the sieve plate plasmodesmata as tubular in nature, Strasburger (1891) questioned this in the gymnosperms. Hill (1908) felt compelled to accept their tubular structure, though he did not picture it in his own illustrations. Schmidt (1917) cast serious doubt on the tubular nature of sieve plate connections, and, with our present progress in microscopic equipment, staining technics, and understanding of protoplasmic properties, it seems certain that these structures are solid. According to present views, the mature functioning sieve tube is a series of elongated cells devoid of nuclei, each lined by a thin parietal layer of protoplasm surrounding a large central vacuole, containing an aqueous solution, within which may be suspended starch grains in varying numbers. Sieve tube elements are connected with neighboring cells by plasmodesmata, and, because of the great affinity of the protoplasm for dyes during certain stages of ontogeny, the connections between adjacent sieve tube elements may be particularly prominent.

Figure 1 shows the plasmodesmata of the sieve plate of squash, and figure 2 illustrates the response of one of these massive, solid connections to cutting the stem. The intense and sudden steepening of the pressure gradient by cutting in the region shown at the lower side of the figure caused one plasmodesma to be forced from its position and inflated within the lower cell. Filled with the slimy sap resulting from disintegration of the nucleus and slime drops, it stains intensely, illustrating by its bladder-like form the fact that the strand from which it was produced was not tubular. These structures have been pictured by Nägeli (1861) and others and occur commonly in cucurbits, ash, and other rapidly exuding plants. Figure 3 shows the long, thin plasmodesmata of the calloused sieve plate of Catalpa and indicates their slender structure and the great elasticity of the protoplasm of which they are composed. Modern microscopic methods, particularly when used upon gymnosperms and many of the lower plants, show definitely that the plasmodesmata between sieve tube elements are not tubular. The thesis that sieve plates are perforate, and thus facilitate mass flow of solution, can no longer be defended.

PHLOEM EXUDATION.—Work by Hartig (1858), Nägeli (1861), Münch (1930), Crafts (1931, 1932, 1936), and Huber, Schmidt, and Jahnel (1937) makes it increasingly evident that mass flow of a sugary solution does occur through sieve tubes, at least in cut stems. These experiments show that phloem exudation occurs in many species, that it takes place at rates well above those normally required to account for growth and storage (Crafts, 1931), and that it may be maintained indefinitely if the phloem is kept open (Crafts, 1936). Linear rates of displacement equivalent to 15 cm. per minute through the sieve tubes have been recorded in cucumber. Exudation was continued for 24 hours in squash by frequent cutting of the stem; and in Macrocystis, which has well developed phloem, plugging is much slower, so that exudation will continue for an hour or more without cutting and can be reestablished by simply cleaning away the mucilage which coagulates on the end of a cut or broken stipe. There can be no question as to the origin of this exudate, since there is no xylem in Macrocystis, and the sieve tubes of the phloem are the only specialized elements capable of such rapid conduction. Three other species of kelps also exuded copiously from the phloem.

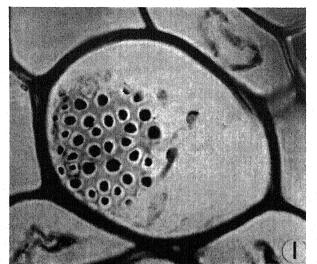
Calculations on the rate of sap displacement in exudation experiments show the values presented in table 1. It is evident from these figures that. since the length of sieve tubes having their contents displaced exceeded the stem length (Crafts. 1936), elements other than those actually cut are involved; in fact, the only logical interpretation seems to be that the exuded solution is moving from considerable distances along permeable elements. Slime plugs (fig. 4) may be found in the sieve tube elements of cucurbits for a distance of several centimeters back from the cut end if the stem is killed in hot water or alcohol. If 1 cm. portions are cut from the stem and killed in alcohol, these structures may be found on the distal sides of the sieve plates at both ends of the section, and in some place between the ends the situation pictured in figure 5 may be found. Here the slime accumulations in adjacent cells are pointed in opposite directions. the plasmodesmata of the sieve plate were tubular, the slime plugs of figure 4 would not occur. It would likewise be difficult to account for their formation if there were no flow of solution through the cytoplasm covering the end wall.

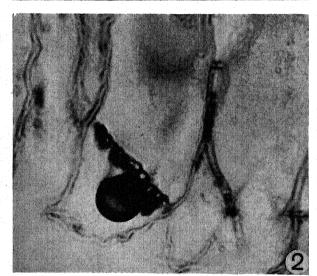
If phloem exudation is causally related to phloem conduction and dependent upon a permeable state of the sieve tube protoplasm, it should be demonstrable in all actively assimilating plants. A simple test has shown that it is. When the rapidly assimilating shoot of any plant is excised and the diagonally-cut end immediately placed in a tube of water, careful observation will show a narrow column of dense liquid descending from the phloem region. Observation of the descending stream depends upon the difference in refraction caused by the density gradient at its boundaries. This test has been conducted on more than 60 species, to date, and has never failed when active shoots were

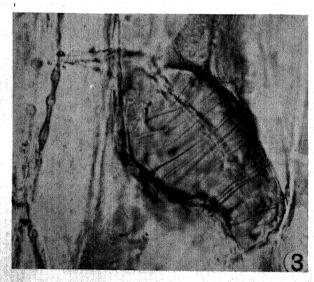
Refinement of the technic of Münch has further shown that phloem exudation can be demonstrated quantitatively in most woody species. Active phloem exudation has been confirmed in white pine and other conifers where plasmodesmata in the sieve areas are notably slender. Huber, Schmidt, and Jahnel (1937) have recently given an excellent picture of concentration gradients correlated

Table 1. Sap displacement rates in pumpkin, cucumber, squash, and kelp.

	Displacement based on		Duration Tissue removed of the by repeated		
Plant used	Phloem	Sieve tubes	experiment		Citation
Pumpkin	13.7 cm.	68.5 cm.	21 min.	1.0 cm.	Crafts (1931)
Cucumber	27.4 cm.	82.2 cm.	16 min.	1.6 cm.	Crafts (1932)
Squash	173.0 cm.	519.0 cm.	12 hrs.	36.0 cm.	Crafts (1936)
Squash	189.9 cm.	569.7 cm.	24 hrs.	72.0 cm.	Crafts (1936)
Kelp	63.4 cm.	253.6 cm.	30 min.	3.0 cm.	Crafts (June, 1938)











overemphasized.

with the height of sampling in a number of trees.

MECHANISM OF SOLUTE MOVEMENT.—We are now faced with the problem of explaining a demonstrated exudation through elements having no intervacuolar pores. The following solution, related to the physical properties of sieve tube protoplasm and intimately connected with phloem ontogeny, is

proposed.

A reconnaissance of phloem ontogeny in many species emphasizes the fact that the young sieve tube element, rapidly expanding under a high turgor pressure, is a nucleate cell with active streaming protoplasm surrounding vacuoles of high osmotic concentration. These cells have the ability to accumulate vital stains and plasmolyse normally in hypertonic solutions. At maturity the nucleus of the element expands, becomes diffuse, disintegrates, and is lost as a discrete entity. This fact cannot be

Accompanying nuclear disintegration, the cytoplasm ceases streaming, loses its clear distinct inner phase boundary, becomes thin, fibroid, elastic, and heavily staining, and fails to plasmolyse in hypertonic solutions. Having lost its ability to retain solutes within its vacuoles, it assumes a truly parietal position and becomes permeable to solutes as are the walls. In this condition it persists throughout the period in which the sieve tubes function in rapid longitudinal conduction. At death the protoplasm and definitive callus are dissolved, and air enters the flattened or crushed elements.

In 1931 the above facts were reported, backed by only a few isolated observations. Within the past six months, however, a survey covering some 58 species of widely diverse character has shown that the most constant features of the mature sieve tube cell are: (1) it is enucleate; (2) it does not accumulate vital stains; (3) it fails to plasmolyse; and (4) being almost devoid of stainable contents, it is clear and translucent in appearance. These features clearly indicate that the mature sieve tube element becomes highly permeable, an interpretation that has recently been confirmed by Huber and Rouschal (1938).

Table 2 lists the genera, one or more species of which have been tested for sieve tube permeability. In these tests longitudinal tangential sections of living stems were cut through the phloem and cambium. These sections, usually 40 to 80  $\mu$  in thickness, were placed in dilute neutral red in M/80

Table 2. Genera in which protoplasmic permeability was tested by means of neutral red staining and plasmolysis.

Macrocystis	Gnetum	Robinia
Polytrichum	Zea	Allanthus
Psilotum	- Cordyline	Acer
Lycopodium	Dioscorea	Aesculus
Selaginella	Iris	Tilia (2 sp.)
Equisetum	Casuarina	Gordonia
Pteris	Populus (2 sp.)	Melycitus
Polystichum	Juglans	Eucalyptus
Woodwardia	Beta	Aralia
Cycas	Cercidiphyllum	Griselinia
Podocarpus	Magnolia	Arbutus
(2 sp.)	Drimys	Frazinus
A raucaria	Illicium	Solanum
Sequoia	Liriodendron	Nicotiana (2 sp.)
Libocedrus	Hedycarya	Catalpa
Abies (2 sp.)	Laurelia	Cucumis
Pinus	Sassafras	Cucurbita
Ephedra	Liquidambar	Helianthus
• •		

borate buffer solution at pH 7.8 (Bailey and Zirkle, 1931). After allowing time for accumulation of the vital stain, the sections were mounted and studied under the microscope. Having selected a favorable location on the section, including, wherever possible, both young and mature sieve tubes in an uninjured condition, the mounting solution was replaced by hypertonic sucrose solution. This was accomplished under observation by adding the sucrose solution on one side of the cover glass and withdrawing the mounting solution from the other side by means of filter paper. The clear, translucent appearance of mature sieve tubes, their paucity of stainable contents, and their failure to plasmolyse constituted their most characteristic properties.

These common features of sieve elements, constant throughout many plants, apparently characterize phloem conductors in all plants and are of diagnostic value in identification of vascular tissues in fossil remains. They should aid materially in building a clearer concept of the origin and extent of vascularization of plants. Added to the evidence on protoplasmic connections and phloem exudation, these facts seem to favor the mass flow interpretation of phloem conduction.

Discussion.—Much of the argument for the protoplasmic theories rests upon alleged evidences for independent movement of solutes in the phloem.

Fig. 1-5.—Fig. 1. Transverse section of squash, showing a portion of a sieve plate with dense, solid protoplasmic connections, light-colored callus cylinders through a gray cellulose matrix. ×700.—Fig. 2. Longitudinal section of squash, showing a bladder-like structure formed by the pressing through and inflation of one of the connections of the sieve plate. Found near the cut end of a rapidly growing young stem. ×700.—Fig. 3. Heavily calloused sieve plate of Catalpa, as shown in longitudinal tangential section. Protoplasmic connections are stretched to many times their original length. ×700.—Fig. 4. Slime plugs near cut end of young, rapidly growing squash stem. These structures consist of colloidal products of the disintegration of the slime drops and nucleus filtered from the flowing sap by the parietal cytoplasmic layer of the sieve tube element. ×350.—Fig. 5. Slime plugs as in figure 4 may be found on the distal sides of sieve plates at both ends of a one-centimeter section of squash stem. Near the center of the stem the contents are less densely accumulated, and in one region of each phloem strand the condition shown here is found. This figure shows the region from which sap flowed in opposite directions to the two ends of the stem section. ×250.—Figures 1, 2, 4, and 5 from a slide by Dr. Esau.

Yet, according to a recent analysis (Crafts, 1938), despite many attempts (Phillis and Mason, 1936; Palmquist, 1938), there has not been described a single critical experiment proving rapid independent movement in the sieve tubes. In the meantime, C. W. Bennett (1934, 1935, 1936, 1937) has built a consistent picture of the conduction of curly-top virus in the sugar beet that can be explained only on the basis of a correlated movement of virus and assimilates by mass flow. With no adequate demonstration of independent movement of solutes at rates of 20 cm. per hour or more through the phloem, experiments on translocation can be explained more logically on the basis of mass flow.

Insufficient attention has been given the fact that the functioning sieve tube element is devoid of a nucleus. The profound changes in the cytoplasm that result from this condition have been largely overlooked (Schumacher, 1933; Mason and Phillis, 1937). The whole history of the sieve tube portrays the intimate relation of the nucleus to the structure and function of the elements. No student of ontogeny can fail to sense the influence that the loss of nucleus has upon subsequent activity. From the beginning of its functioning period to its death, the sieve tube element is doomed to a passive rôle, conditioned by its lack of nucleus and consequent permeability. The only remaining evidences of life are the maintenance within the vacuole of a fluid state, the continued development of callus to its ultimate definitive condition, and a peculiar responsiveness to stains (Schumacher, 1930).

Until the proponents of the protoplasmic theories can produce some concrete evidence for protoplasmic streaming or a high activity state in the protoplasm of the mature sieve tube, there is no bridge for the obvious gap between their experimental data and their final interpretation. Studies on sieve tube ontogeny, on phloem exudation, and on virus movement all point to a mass flow mechanism.

### SUMMARY

Careful studies using modern microscopic equipment and improved technics show that the plasmodesmata of sieve plates are solid and not tubular in structure.

Experiments indicate that phloem exudation occurs in many plants, that it takes place at rates above those required to account for growth and storage, and that it may be maintained indefinitely. Slime plugs of particulate or colloidal material are formed by the filtering out of sieve tube contents by protoplasm on the sieve plates. They indicate a mass flow of sap through the lumina.

A simple technic using the difference in density between phloem exudate and water has revealed phloem exudation in all species tested. Refinement of the Münch technic has given a quantitative demonstration of phloem exudation in many woody species, including white pine.

A reconnaissance of phloem ontogeny in many species emphasizes that, whereas the young sieve tube is a nucleate cell with active streaming protoplasm surrounding vacuoles of high osmotic concentration, the mature element is enucleate and fails to plasmolyse in hypertonic solutions. It is assumed that this indicates a high state of permeability in mature sieve tubes.

Tests on 58 species, ranging from the mosses and giant kelps to the most highly specialized angiosperms, show that this permeable state of the mature sieve tubes may characterize these elements in all plants. The common occurrence of phloem exudation and permeable sieve tubes supports the pressure-flow mechanism of the translocation of organic solutes in plants.

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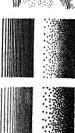
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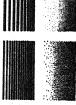
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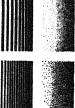
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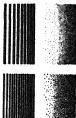
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### DRAWING GUIDE FOR ZINC ETCHINGS

Letters, lines, and dots in relation to reduction for the printed page. Top-Reduction to 1/4. Middle-Reduction to 1/2. Bottom-Original size. Reprinted by permission of the authors from: Riker, A. J., and REGINA S. RIKER. 1936. Introduction to research on plant diseases. 117 p. Published by the Authors, Department of Plant Pathology, University of Wisconsin, Madison, Wis.

blotches when the drawing is reduced. Keep the shad-Note that thin black lines hold up fairly well in reduction, but that small black dots and small white spaces between black lines or dots may be lost. If placed too close together, dots and lines produce black ing rather open. The degree of reduction needs to be known before the drawing is inked in,

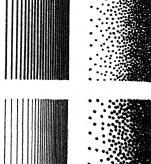
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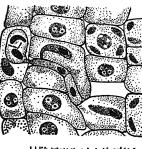
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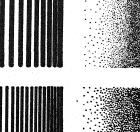
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### A DEVELOPMENTAL ANALYSIS OF THE RELATION BETWEEN CELL SIZE AND FRUIT SIZE IN CUCURBITS!

Edmund W. Sinnott

THE PROBLEM of the relation between cell size and organ or body size in plants has been extensively studied and forms the basis of a considerable literature, but the lack of any general agreement in the results obtained indicates that the question is not a simple one. Sachs (1893) and Amelung (1893) presented evidence that size differences in body and organ are due primarily to differences in the number rather than in the size of their constituent cells, and this conclusion has been frequently confirmed. Other workers, however, among them the present writer (1930), have found that in many cases differences in organ size are in part, at least, related to cell size. Almost all the evidence has been gathered from a study of organs and cells which had reached maturity. Both structures attain final size, however, as the result of growth from small beginnings; and a developmental analysis of the growing organ in terms of changes in the size and in the number of its cells should yield important results. A study of the whole developmental process, with particular reference to the relative rates and durations of cell division and cell expansion, is evidently needed as a fundamental approach to the problem. Such studies have been made in only a few cases. Notable among these is the work of Houghtaling (1935) on tomatoes, in which she showed that early fruit growth is due chiefly to cell multiplication and later growth entirely to cell expansion and that the extent of both these processes was greater in the larger fruits than in the smaller ones. Similar results have been reported by MacArthur and Butler (1938).

The present study was undertaken to determine the developmental relations between cell size, cell number, and ovary and fruit size in several genera of the Cucurbitaceae and particularly in various races of Cucurbita Pepo differing markedly in fruit size.

The writer wishes to express his sincere thanks to Mrs. Helen Houghtaling Kaltenborn, Mrs. Mary Roscoe Vestal, and Miss Elizabeth Lane Toole, who have been associated with him at various times in the study of this problem, for their skilful assistance in the gathering and analysis of data and for many helpful suggestions. For financial support of this investigation he is greatly indebted to the Fund for Research of Columbia University.

MATERIALS AND METHODS.—Ovaries and fruits of the genera Cucurbita, Lagenaria, Cucumis and Citrullus were studied. Cucurbita Pepo provides particularly favorable material because of the great variation in fruit size between races of this species. The forms studied were members of inbred and es-

<sup>1</sup> Received for publication February 8, 1939.

sentially pure races. The twelve races analyzed are as follows: in Cucurbita Pepo, Race CF, from the "Connecticut Field" type of pumpkin, about 300 mm. in diameter; Race MT, from the "Mammoth Tours" type of pumpkin, about 360 mm. in diameter; Race M35, from the "King of the Mammoths" type of pumpkin, more than 300 mm. in diameter; Race 103, a segregated, nearly spherical race about 120 mm. in diameter; Race SRC, a Chinese gourd, about 60 mm. in diameter; Race SP, from a "spoon" type of gourd, about 45 mm. in diameter; and Race TA, a small, pear-shaped gourd about 40 mm. in diameter. In Lagenaria vulgaris, Race GB is from a large "Bottle" type of gourd, about 170 mm. in diameter; Race CN is from the "Crane's Neck" type of gourd, more than 100 mm, in diameter, and Race CL from the "Club" type of gourd, about 100 mm. in diameter. In addition to these there were studied Race G, from the West Indian gherkin, Cucumis Anguria, about 30 mm. in diameter; and Race WM, a variety of watermelon, Citrullus vulgaris, about 300 mm. in diameter. In Cucurbita the largest type thus has fruits more than 700 times the volume of the smallest, and the large pumpkin is considerably more than 1,000 times the volume of the gherkin.

The plants were grown in the field, at the same place, and under as nearly uniform conditions of soil and culture as possible. Material was collected at many stages from very small ovaries to mature fruit. The smallest were from 1-2 mm, wide, the size at which the ovary becomes organized as such and can readily be measured. The two major dimensions were measured; width at the widest point and length from the point where stalk and ovary join to the constriction between ovary and calyx lobes. With small ovaries the entire organ was collected, but in larger ones a transverse slice several millimeters thick was taken across the ovary at its widest point. In large ovaries and fruits, a segment of this region from the outside to the center, or samples of the various tissues in such a segment, were collected. Part of the material was killed in formol-aceticalcohol, part in Carnoy's solution, and part in Craf, the last giving the best results. Material was embedded in paraffin and transverse and longitudinal sections cut. Haematoxylin stains, chiefly Heidenhain's, were used, since they best brought out the cell walls.

Cell diameter in the transverse plane was especially studied and was compared with organ diameter in the same plane. Since the cells in the tissues studied were found to be essentially isodiametric, cell volume could be determined in relation to fruit volume; but the essential facts were obtained most simply by a study of transverse diameters.

Anything like an accurate measurement of cell size in a mass of tissue is beset with difficulties, which the writer does not wish to minimize; but if care is used in obtaining data, if large numbers of cells are measured, and if the results are consistent and fall into a fairly simple system, the investigator may be confident that he is not far from the truth. The method used in the present study was to draw cells with a camera lucida or a projector at known magnifications and to measure the drawings, computing from these the actual sizes. The most difficult problems here were to determine where to make the measurements and which cells to measure. In all races there is a gradient in cell size from the inside of the ovary outward, the cells growing progressively smaller toward the epidermis. Measurements were made at a number of points along this gradient. Several regions may readily be distinguished:

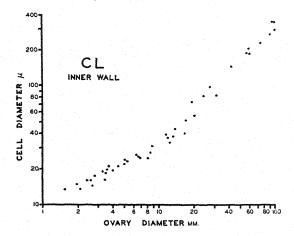


Fig. 1. The relation of cell diameter to fruit diameter in the inner wall of Race CL. Each point represents the average of 20 measurements. Both variables are plotted logarithmically.

the central placental region, normally consisting of three ovule-bearing sections; the inner wall, between the outside of the placental region and the ring of twenty main vascular bundles; the outer wall, between these bundles and the epidermis; and the epidermis itself. In all the genera except Lagenaria, two definite layers can be distinguished in the inner wall: an innermost one in which the phloem strands run lengthwise of the ovary, and an outer one where they run at right angles to this, or tangentially. These layers are called the "inner" and "middle" layers in these types. The morphological nature of the peripheral tissues in the ovary and fruit of this family seems not to be well established and for this reason the non-committal word "wall" is here used.

Definite points were chosen in these various layers at which cell sizes were measured. These were as follows: (1) the area near the axis of the placental region, just outside the point where the three carpels come together; (2) an area half way radi-

ally across the inner wall; (3) an area half way across the middle wall; (4) an area half way across the outer wall; and (5) the epidermis. In Lagenaria, of course, there were only four points of measurement since here (2) and (3) are indistinguishable. In this genus the region chosen here was half way from the placenta to the bundles. In most races cells were also measured in the region between two bundles and thus intermediate between the middle and outer walls; and also in the hypodermal layer, just under the epidermis. For a number of reasons uniform measurements were difficult to make here, however, and the data are not included in the present paper.

Even in these very definite regions there are considerable variations in apparent cell size. Some of these are due to the fact that the cells have been cut at various levels and thus appear to have somewhat different diameters. A much more important cause, however, is that throughout early developmental stages cell division is taking place, so that small cells, resulting from recent divisions, are intermixed with larger ones which have expanded considerably. In all tissues, however, if occasional abnormally large cells are disregarded, a common maximum size can be distinguished which is characteristic of the whole area and is apparently the cell size at which, in this region, division will again take place. These are the cells which were chosen for measurement, and with a little practice they can rather readily be picked out. For each of the five (or four) tissue regions in each ovary studied, twenty of the typical cells were drawn on a single sheet. Among these the ten least extreme in size and shape were chosen, and in each of these the longest and shortest diameters were measured. The average of the twenty measurements was then taken, after correction for magnification, as the cell diameter for this tissue. The fact that cell measurements made in this way by four persons yielded very similar and consistent results indicates that the method is a dependable one and that the data are trustworthy.

The most satisfactory method of analyzing the relationship between cell size and organ size during development is to plot one variable against the other at as many stages in growth as possible. Since growth is an exponential process, it is much more satisfactory to plot the logarithms of these values rather than the values themselves. This makes it possible to compare small and large members of a series on an equal basis. In practice, the values were plotted on double logarithmic paper, with cell diameters on the ordinates and ovary diameters on the abscissae.

RESULTS.—General relations of cell size to organ size.—In figure 1 are shown the data, plotted on a double logarithmic grid, for the inner wall of Race CL, a "Club" type of Lagenaria. Each point represents the average of twenty measurements of cell diameter in this tissue, in an ovary of the diameter indicated. It will be observed that from the smallest sizes, with an ovary diameter of about 2 mm.,

these points form a rather narrow band which is essentially linear and which rises with a slope of about 45 per cent. This indicates that cell diameter increases as ovary diameter increases, but only about 45 per cent as fast. In other words, the value of the relative growth constant (a in the allometric formula of Huxley and Teissier, 1936) is .45. In ovary diameters greater than 12 mm. this relationship changes markedly, the band bending upward sharply to a value of about 1.0, which is maintained to the end of growth. Evidently after the attainment of an ovary diameter of 12 mm., cell diameter and ovary diameter are increasing at equal rates. To facilitate more exact comparison of these growth relationships, a line may be drawn through the center of the band. It will consist of two linear portions meeting at the point of inflection. This has been done in all later figures.

The fact that in early development ovary width is growing faster than cell width can be explained only by assuming that cell number must here be increasing. Since beyond the point of inflection both cell and organ increase equally, there is evidently no increase in cell number here. The inflection point thus indicates (subject to a correction discussed below) the approximate ovary and cell diameter at which, in this tissue, cell division ceases. Before this point, growth is the result both of cell multiplication and of cell enlargement. After this point is reached, it is the result of cell enlargement only.

Two features of this developmental relationship between cell size and organ size are noteworthy. First, there is evidently a considerable increase in cell volume even during the period when cell division is going on. This must mean that there is no fixed size at which cell division occurs but that after each successive division the two daughter cells, before they themselves divide again, increase to a size considerably greater than that of their mother cell at the time of its own division. Second, the fact that the inflection point is sharply marked and that the line leading up to it is straight rather than curved indicates that division stops suddenly rather than gradually, and that the rate of division is constant and is independent of the size of the cell. Thus cells with a volume of 30,000 cubic micra apparently divide as rapidly as those of 1,000 cubic micra, a rather remarkable fact, since larger cells might be supposed to divide less rapidly than smaller ones.

The cessation of division is associated with the attainment of a definite cell size. This size is evidently a matter of some significance in the developmental process. It is evidently not exactly the size at the inflection point but a somewhat lower value. Increase in cell size is not a continuous process but is interrupted at each cell division, for cell volume is then reduced by half and cell diameter by slightly more than 20 per cent (logarithmically by approximately .1). Each daughter cell now increases (at the same rate as the whole tissue) to a somewhat greater diameter than that of its mother cell and then divides again. Thus the changes in size in a given cell

lineage are not to be represented by a continuous line but by a serrate one (fig. 2). The line shown in the figures in the present paper is that which connects the tips of these "teeth," since we have endeavored to measure the cell diameter at its normal maximum before each division. After the last division, cell enlargement proceeds continuously, uninterrupted by further breaks, at approximately the same rate as fruit enlargement. The cell diameter at the last division is therefore that at the tip of the

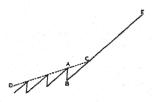
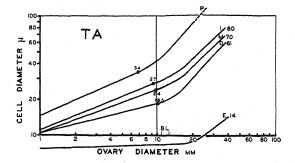
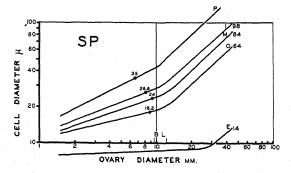


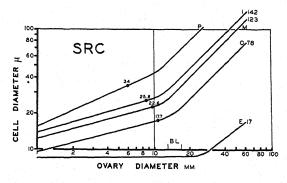
Fig. 2. Diagram showing how cell diameter (ordinates) changes in relation to ovary diameter (abscissae) in a single cell lineage for a given tissue. A. cell diameter at the last division. B. diameter of a daughter cell at its formation. C. inflection point. The line D-C-E represents those shown in fig. 3-14.

last "tooth," or the point at which the diameter of a cell half as large (and thus with a diameter slightly less than 80 per cent as great) will fall on the downward prolongation of the second part of the growth line, after the inflection point. Thus in figure 2, C is the inflection point, A the diameter at the last division, and B the diameter of the last daughter cell at its formation. This cell now becomes greatly enlarged. This method of determining the cell diameter at the last division can be checked by direct observation. Mitotic figures are not numerous, but they can be found, and the largest cells in which these occur, in a given tissue, are of approximately the size thus calculated. Furthermore, tissue in which division is still going on can usually be recognized by the occurrence within it of obviously new cross walls separating what were the two halves of a recently divided cell; and the point at which division ceases, as indicated in this way, gives a good general agreement with the other methods.

For the twelve races analyzed, the relation of cell size to ovary size was determined for each tissue and from small ovaries to mature fruits, and the results were essentially the same as in the example just described. A line was drawn through the middle of the band of points in each tissue, and the lines for the various tissues in a given race were all plotted on the same double logarithmic graph. The results are presented in figures 3 to 14. In a few, analysis begins with ovaries of 1 mm, in width, but in one the smallest was 2.5 mm. The average diameter at the start of observation was somewhat less than 2 mm. The cell diameter at which the last division occurs, as determined by the method first outlined above, is shown by a black circle, and the value in miera indicated above or beside this. The cell diameter at maturity, in micra, is also given. The lines for the pla-







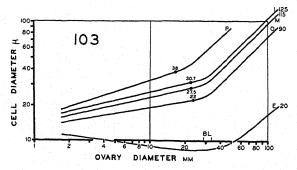


Fig. 3-6.—Fig. 3 (top). Cucurbita Pepo, Race TA.—Fig. 4 (second). C. Pepo, Race SP.—Fig. 5 (third). C. Pepo, Race SRC.—Fig. 6 (bottom). C. Pepo, Race 103. Graphs showing relative growth of cell diameter to ovary diameter for all tissues in the twelve races are shown in fig. 3-14. Each line has been drawn through the middle of a band of points similar to that shown in fig. 1. Solid circles, cell diameter at last division. Values given are for cell diameters in micra at the last division and at maturity. P, placental region. I, inner wall. M, middle wall. O, outer wall. E, ep.dermis. BL, flowering size.

cental region, inner wall, middle wall, outer wall, and epidermis are indicated by the letters P, I, M, O, and E, respectively. Cell diameter at any fruit diameter may be approximately determined from these graphs. In table 1 are given the actual values for the cell diameters of the various tissues at two convenient ovary diameters (2 and 10 mm.), at the point of last division, and at maturity. The relative growth constants for cell diameter against ovary diameter, during the period of cell division, are also given.

Differences between tissues.—A study of the developmental cell-organ size relationship in these twelve races shows that in all of them the various tissues (except the epidermis) behave in essentially the same way as in the example given above for the inner wall of Race CL, a period of cell division and slight expansion changing rather suddenly to one of expansion only. Although this is the general procedure, there are certain consistent differences between tissues in the same race, and between different races, which are significant.

The most obvious difference between tissues is in the rate at which cell enlargement takes place during the period before division ceases. Since the lines for the various tissues diverge, under logarithmic plotting, it is evident that the cell size differences between them are becoming not only actually but relatively greater. In other words, differentiation is occurring. Thus between an ovary width of 2 mm. and 10 mm., the placental cells on the average increase in diameter about 100 per cent but those of the outer wall only about 30 per cent. Furthermore, in every race there is a gradient in cell increase, each successive tissue, from within outward, showing a lesser rate of increase than the one inside it. In races where measurements were made for the bundle region and for the hypodermal region, these were found to occupy their expected positions in this gradient-namely, between the values for the middle and outer wall and between those for the outer wall and epidermis.

A somewhat similar gradient exists in the time at which cell division ceases. This typically occurs first in the placental region and successively later in the more outer tissues. The difference between the tissues in this respect is much greater in some races than in others, but the order is the same.

A third difference is in the cell size at the last division. This is typically greatest in the placental region and successively less in the outer ones. In a number of instances these outer tissues continue to divide until they approach the same cell size that the placenta had at its last division, but some difference always remains.

The epidermis is the most extreme of the tissues in all three of these respects. Its cells increase but little in size and in some races may actually decrease for a time. The inflection point occurs very late, showing that division persists much longer than in other tissues. Cell size at the last division is very small. The inflection point is not sharply marked,

as elsewhere, and the slope of the line following it often does not reach a value of 1.0 for some time, so that cell division evidently does not cease here as suddenly as in the other tissues. The dimension of the epidermal cells here given is the periclinal (tangential) one, which is essentially the same as the longitudinal dimension, since the cells, seen in surface view, are isodiametric. The anticlinal or radial dimension usually follows about the same course as the periclinal but is somewhat greater in absolute size. It pursues a slow but steady increase without a marked inflection point, doubtless because cell division does not occur in the tangential plane and the "saw tooth" type of growth is absent.

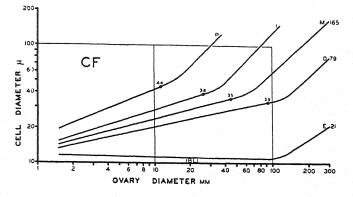
In all tissues the second growth period, in which cell expansion alone is occurring, shows an approximately equal growth rate of cell and ovary, although this varies somewhat, since the various tissues do not grow at the same rate as the ovary as a whole. In the innermost tissues, especially of large-fruited races, the cells die and collapse before fruit maturity is attained.

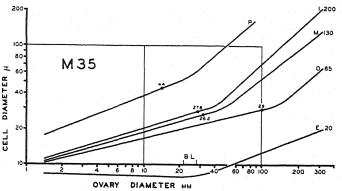
It will be observed that the lines for all the tissues of a given race, if projected backward, tend in most cases to converge to a common point of origin. This is usually at a cell diameter in the vicinity of 10 micra and an ovary width of something less than 1 mm. This cell dimension is not always that of the actual meristem, but of the ovary primordium when differentiation among its tissues begins. The early history of the ovary, reconstructed in this way, is only an approximation, for at smaller sizes than 1 mm. analysis is difficult.

Differences between large-fruited and small-fruited races.—The general relation between cell size and organ size during development is much the same in the twelve races studied, but there are a number of significant differences observable which throw some light on the developmental mechanism by which fruit growth is accomplished.

These differences can best be studied in the seven races of Cucurbita Pepo. Three of these, TA (fig. 3), SP (fig. 4), and SRC (fig. 5), have small fruits, from 40-65 mm. wide. Three others, CF (fig. 7), M 35 (fig. 8), and MT (fig. 9), are much larger, with fruits over 300 mm. in diameter. Race 103 (fig. 6) is intermediate in size. The analysis of these races as shown in the graphs makes it possible to compare the development of a small fruit with that of a large one in at least six respects, as follows:

(1) It is clear that there are no significant differences in initial cell size in the young ovary between large-fruited and small-fruited types. At an ovary diameter of 2 mm., the former have an average cell





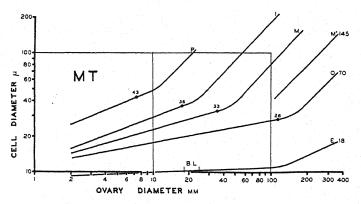
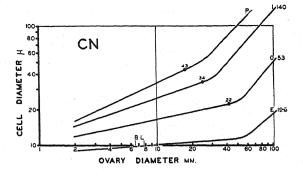
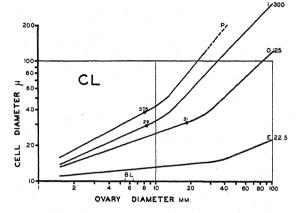


Fig. 7-9.—Fig. 7 (above). C. Pepo, Race CF.—Fig. 8 (center). C. Pepo, Race M35.—Fig. 9 (below). C. Pepo, Race MT.

diameter for all tissues about 5 per cent greater than the latter. At an ovary diameter of 10 mm., however, the small races have cells about 2 per cent wider than the large ones. Cell size at the meristem has not been studied, but it seems doubtful if there is any fundamental difference here.

(2) During the period of cell division, the small-fruited races have a generally higher relative rate of cell enlargement compared to ovary growth, as is shown by the somewhat steeper slope of their lines. For every tissue, the average value of this relative growth constant is higher for the three small-fruited races than for the large ones, the general average for the former being 34.7 as against 30.9 for the





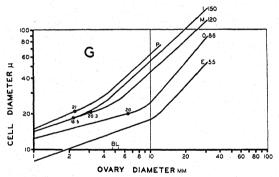


Fig. 10-12.—Fig. 10 (above). Lagenaria vulgaris, Race CN.—Fig. 11 (center). L. vulgaris, Race CL.—Fig. 12 (below). Cucumis Anguria, Race G.

latter (table 1). This means that a given cell size is reached at a somewhat smaller ovary size in the small-fruited types than in the large ones. If cell division ceases at the same cell size, it will therefore evidently be prolonged somewhat in ovaries of the large types, a larger number of cells will be produced, and a correspondingly greater fruit size attained at maturity. It can readily be calculated that the increase in fruit diameter due to this cause alone is about 30 per cent.

(3) Another difference between large and small types is the fact that in the former, the cell size at which division ceases is considerably greater than in the latter. For the four tissues in which it was determined, the average cell diameter at the last divi-

sion was 34.75 micra for the large-fruited races and 25.63 micra for the small ones, indicating a difference of more than two and a half times in volume. This means that cell division persists markedly longer in the former than in the latter, with a corresponding increase in cell number and thus in fruit size. An ovary with the final cell size which is the average for the three large races would thus have a diameter, due to this plus the relative cell-organ growth rate difference, more than three and a half times that of one with the average values for the three small races, and would have a volume of ovary and fruit more than 45 times as great.

(4) The situation is complicated, however, by a fourth difference between large and small fruitsnamely, the total duration of cell division. Division ceases first in the inner tissues and later in the successively outer ones; but in the small-fruited types this all occurs within a relatively short period. whereas in the large ones it is spread out over a much longer one. Thus in Race TA, division ceases in the placenta at an ovary diameter of 6.9 mm. and in the outer wall at 10 mm.; in SP these values are 6.5 and 9.0, and in SRC, 6.0 and 10.5 mm. Division thus ceases rather abruptly, except in the epidermis. In CF, on the other hand, division in the placenta ceases at 11.5 mm. and in the outer wall at 92; in M35 the range is from 14.5 to 100, and in MT from 7.3 to 115 mm. The period is over five times as long, logarithmically, in the large races as in the small ones. In the former the various tissues tend to approach the same cell size before division ceases, although this is never quite attained. In the latter there are markedly greater differences in cell

This much greater duration of cell division, and the consequently larger number of cells produced, is evidently a major factor in determining the greater fruit size of the large types. It might be thought possible to determine the actual increase in cell number for each race by dividing organ size by cell size in the various tissues. This cannot be done accurately from the data here presented, however, since the various tissues are increasing at different rates and since cell size should be compared with its particular tissue volume to make a calculation of cell number possible.

size when division ceases.

(5) The amount of cell expansion, following the cessation of cell division, tends to be considerably higher in the large-fruited types. In all races the placental cells, which are the largest from the start and begin to expand first, collapse before fruit growth ceases. The relatively very great enlargement which they would undergo, if they continued to expand to the end, is too much for their powers of enlargement, and they die, become filled with air, and finally are pulled apart and disintegrated by the continued growth of the outer layers. In large types the whole inner placental region disappears, and the mature fruit is hollow.

The wall layers all survive to maturity in the small types, but in the large ones the inner wall usu-

ally breaks down and sometimes the middle wall as well. This is due to the fact that division extends over such a long period here that the inner layers have to expand very greatly if they are to persist. In Race MT there arises around each phloem strand in the middle wall a group of small cells. As the primary cells of the wall enlarge and collapse, these secondary ones expand and fill their place. This produces the stringy, fibrous character of the wall evident in coarse-fruited types of this sort, as compared with the much more homogeneous character of fine-textured races like M35.

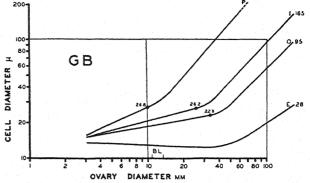
Race M35 is the only large type in which cell size can be compared with the small-fruited races. It is evident that in inner and middle walls its cells are much larger. Even though the inner lavers in the other races do not persist to maturity, they expand considerably further before collapsing than the final size in the small-fruited types. The outer wall in the large types, doubtless since it begins its expansion so late, usually does not have larger cells than those of the small races. The epidermal cells are considerably larger, however. It is clear that large fruits have consistently larger cells at maturity than small ones and that this is due partly to larger cell size when division ceases, but chiefly to the greater expansion which their cells then undergo.

The important part which cell size differences often play in determining

fruit size is also well shown by a comparison of the three small races. They have very similar developmental schedules up to the time when division ceases but vary considerably in the amount of later expansion, TA having least, SP next, and SRC most. The final fruit size is almost directly proportional to the amount of cell expansion, and difference in fruit size among these races is therefore related primarily to differences in cell size rather than in cell number. TA with as much cell expansion as SRC would produce a very similar fruit.

(6) Another point of general difference between large and small types is in ovary size at flowering. This is consistently larger in the large types, with an average diameter of about 22 mm. as compared with about 11.5 mm. in the small ones. Thus at a comparable stage of development the large types have larger ovaries, with larger cells and a higher degree of differentiation. At comparable stages from the beginning, the large-fruited types are somewhat larger.

Race 103 occupies an intermediate position between the large and the small races, in mature fruit size. This seems to be due primarily to the fact that in early development cell enlargement is relatively



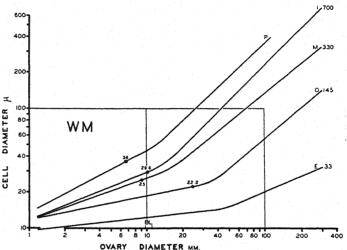


Fig. 13-14.—Fig. 13 (above). Lagenaria vulgaris, Race GB.—Fig. 14 (below). Citrullus vulgaris, Race WM.

slow as compared with cell multiplication, so that the relative growth ratios of cell to ovary diameter are the lowest of any race. This, together with a slightly higher cell size at the last division, results in a very large number of cells. Race 103 in other respects resembles the small races, for cessation of division in all tissues occurs almost simultaneously, and the amount of cell expansion afterward is relatively very small. In its earlier development, 103 resembles the large-fruited races; later, it resembles the small ones. Evidently the various elements determining final fruit size are independent of each other and may be combined in various ways.

Other genera.—The three races of Lagenaria vulgaris analyzed differ rather markedly among themselves.

Race CN has a relatively large cell size when division ceases and a long period of cell multiplication, but the extent of later cell expansion is relatively slight so that final fruit size and cell diameter are small. Like Race 103, in early development this resembles a large-fruited race and later a small one. Conditions are exactly reversed in Race GB, for here the cell size when division ceases is usually small so that the duration of cell multiplication is less; but the amount of expansion is great, with the

Table 1. Cell diameters in micra for the various tissues of the twelve races, at ovary diameters of 2 and 10 mm., at last division, and at maturity; and relative growth constants of cell diameter to ovary diameter for each tissue in early development. P, placental region. I, inner wall. M, middle wall. O, outer wall. E, epidermis.

Race and mature fruit diameter	Tissue	Cell diameter, 2 mm.	Cell diameter, 10 mm.	Cell diameter, last division	Cell diameter, maturity	Relative growth constant, cell to ovary
	ſP	20.0	40.0	34.0		.430
	I	14.5	27.8	27.0	80	.402
TA, 39 mm	. ∤M	13.0	24.2	24.0	70	.369
	0	12.6	18.5	18.5	61	.235
	E	8.0	8.3		14	.028
	P	19.5	43.0	35.0	•	.513
	I	15.5	29.0	26.8	98	.390
<i>SP</i> , 45 mm	. {M	14.0	24.8	24.0	84	.352
	0	12.8	18.8	18.2	64	.234
	$\mathcal{E}$	8.2	8.7	•••	14	.039
	P	21.2	43.0	34.0		.435
	I	17.1	27.1	25.8	142	.279
SRC, 62 mm	. \M	14.9	23.0	22.6	123	.268
	0	11.5	17.5	17.7	78	.257
	$\mathbf{E}$	8.6	8.8	•••	17	.005
	P	19.2	32.3	38.0		.326
	I	17.5	25.5	30.7	125	.236
<i>103</i> , 120 mm	. \ M	16.2	23.0	27.5	115	.223
	O	14.5	19.0	22.0	90	.169
	E	11.0	9.0		20	124
	(P	21.6	42.0	44.0		.416
	Î	16.8	28.0	38.0		.318
CF, 300 mm	. \\ M	15.5	23.5	35.0	165	.256
, , , , , , , , , , , , , , , , , , , ,	0	14.2	20.0	33.0	79	.216
	E	11.5	11.2	•••	21	017
	P	20.0	38.0	44.0	• • • • • •	.398
	I	12.4	20.0	27.8	200	.304
M35, 315 mm	. \M	11.7	18.6	26.2	130	.292
	0	11.2	16.5	29.0	65	.236
	E	8.3	8.0		20	028
	P	25.0	49.0	43.0		.421
	I	15.8	29.0	36.0		.376
MT, 360 mm	. {M	14.5	23.0	33.0	145ª	.286
	0	13.1	17.7	28.0	70	.191
	E	9.2	9.8		18	.040
	ſΡ	16.0	33.0	43.0	••	.450
	Ĩ	14.5	25.0	34.0	140	.337
CN, 105 mm	$\cdot \mid 0$	12.0	16.5	22.0	53	.202
	E	9.2	10.0		19.5	.056
	ſΡ	13.0	26.8	26.8		
	Î	13.2	20.3	26.2	165	.435
GB, 170 mm	o	14.2	18.5	22.9	95	.270 .174
	E	13.7	12.7		28	047
	(P	18.2	42.0	37.8		
	I				900	.528
CL, 100 mm	O	15.5	32.0	29.0	300	.450
	E	14.4 11.2	25.0 13.1	31.0	125 22.5	.348
				27.0	22.0	
	P	19.7	62.0	21.0		.448
(1 21 mm	I	17.7	54.0	18.5	150	.330
G, 31 mm	M	17.7	43.0	20.3	120	.330
	O	14.7	25.0	20.0	86	.263
	{ <b>E</b>	9.0	17.5		55	.382

<sup>\*</sup> Secondary cells.

Table 1. Concluded.

Race and mature fruit diameter	Tissue	Cell diameter, 2 mm.	Cell diameter, 10 mm.	Cell diameter, last division	Cell diameter, maturity	Relative growth constant, cell to ovary
	ſP	19.0	44.0	36.0		.520
	I	15.2	29.0	29.6	700	.407
WM, 310 mm	∤M	14.8	25.7	25.0	330	.351
	0	13.4	18.5	22.2	145	.203
	E	10.3	12.2		33	.101

result that both fruit size and cell size at maturity are relatively large. GB differs from all other races in the comparatively large ovary size attained (about 2.5 mm.) before differentiation in cell size begins.

Race CL is distinguished by its relatively high rate of cell enlargement in early development, its values being higher (its lines steeper) than in any other type studied. As a result, cell multiplication ceases relatively early, in terms of ovary diameter. and although cell expansion is extensive, the fruit is only a little more than 100 mm. wide at maturity. These facts can be understood in the light of the unusual development of this race, for here, unlike the others, width grows much more slowly than length. Early cell increase is rapid as compared to ovary width, but as compared to ovary volume it is much as in the other races. A given ovary diameter here represents a considerably later developmental stage than in races where length and width increase at nearly equal rates.

Race G, a type of Gherkin, Cucumis Anguria, is the smallest of those studied and has, as might be expected, the lowest cell size at the time when division ceases. Unlike the small-fruited Cucurbita races, however, this size is almost the same for all tissues. Cell multiplication ceases relatively early, and although expansion after division ceases is great, the final fruit diameter is only a little more than 30 mm. Another striking difference between this race and all the others is in the epidermis, which increases steadily from the beginning and has a definite inflection point and a later period of rapid expansion. It thus behaves much like the placental and wall tissues.

Race WM, a watermelon, is chiefly notable for its enormous cell expansion. Even in early development its rate of cell expansion is relatively high, and since cell size at the cessation of division is not great, division stops early. In this respect WM resembles the small-fruited types of Cucurbita. The amount of later expansion is so great, however, that a large fruit is actually produced. The placental cells break down, but the inner wall cells reach the surprising diameter of 700 micra, and in other types even this is exceeded. These large cells seem still to be alive. They have increased to about 350,000 times their meristematic volume.

Discussion.—There are evidently many developmental differences between fruits of different races.

Not only are large-fruited types different from small ones in many respects, but even the same fruit size may be reached in different ways. The determination of fruit size clearly involves many distinct and apparently independent processes. The genetic basis of size differences therefore involves not a single series of growth intensities but many separate physiological processes which seem to be inherited independently. It is not surprising that genetic analysis shows size characters to be dependent on many genes.

The problem of the relation between cell size and organ size is evidently not a simple one but involves all aspects of the growth and differentiation of a determinate organ. It is but one part of a complex developmental problem. The various processes which have been shown here to be concerned in fruit growth will now be more fully discussed.

The rate of cell division and the rate of cell expansion are obviously the two most important of these. No data are here presented as to absolute rates of either, but other evidence, showing that fruit size is not related to growth rate, indicates that there are no very great differences between races in either the frequency of division or the rapidity of expansion. It is clear, however, that considerable differences exist in the relative rates of these two processes, not only between races but especially between tissues, as is shown by the differences in the relative growth constants for cell size against organ size. Where this value is positive the cells increase in diameter, indicating that expansion is more rapid than division, in the sense that the cells expand between divisions to a greater size than their mother cells. An important fact, not sufficiently recognized in discussions of the problem of size, is that a given cell size is merely the resultant of the frequency of cell division as compared with the rapidity of cell expansion. Large cells indicate either fewer divisions, more expansion, or both.

What causes the pronounced differences between internal and external tissues in the relative rates of these processes is not clear. Evidence to be presented elsewhere, however, indicates that rate of cell division is essentially the same in all tissues and that the differences here described are chiefly in rate of expansion. The definite gradient from within outward in this respect suggests a corresponding gradient in respiration rate, osmotic concentration, permeability, pH, or amount of some growth substance.

It is significant in this connection that Gustafson (1939) has recently found a higher concentration of auxin in the central region of a squash fruit than in its wall.

Related to this may perhaps be the other gradient-namely, that in the time of cessation of cell division. This event occurs first in the innermost tissue and progressively later in the outer ones. The facts suggest a gradient in the amount, or in the rate of consumption, of some material essential for cell division which is thus exhausted first in the inner tissues and last in the epidermis. Where this gradient was slight, cessation would occur at almost the same time in all tissues, as was found to be the case in the small-fruited races of Cucurbita. Where it was steep, division in the outer tissues would persist for a progressively longer period, as was found to be true in large-fruited races. These two gradients, whatever the causes for them may be, are evidently of much importance in the problems of both cell size and organ size.

It is evident that there is no fixed cell size at which mitosis occurs, even in the same tissue. Except in the epidermis, successive cell divisions are at progressively larger cell sizes. Division ceases at widely different cell sizes in different tissues. We must look for something other than the attainment of a specific cell size as the factor which initiates mitosis or which finally stops it. Cell size, however, does seem to play some part in the cessation of division, for there is usually a rather definite upper limit beyond which division will no longer take place. After a diameter of about 45 micra, when the cell has become vacuolate, further division is obviously difficult. But the limit is often reached much earlier than this, as in Race G, where in no tissue does it much exceed 20 micra. Perhaps the most remarkable fact, already mentioned above, is that the rate of cell division seems to be the same regardless of cell size. Since the duration of cell multiplication, and thus the number of cells produced, is so closely related to the determination of organ size, the problem of the factors—chemical, physical or otherwise -which initiate or inhibit cell division is evidently of much significance here.

What part the attainment of sexual maturity or flowering may have in these developmental processes is not very clear. In the races of Cucurbita, Cucumis, and Citrullus studied this occurs not long after cell division ceases in the placental region and is perhaps associated with a given stage in the development of the ovule-bearing tissues. This relationship is not so clear in Lagenaria. In the small-fruited races of Cucurbita division in all tissues but the epidermis has just finished when flowering occurs, but in the larger ones, division in the wall layers persists long afterward.

Various factors suggest themselves in explanation of the differences in amount of cell expansion following the end of division. In most types the cells are incapable of living, and their walls of expansion, after a diameter of about 200 micra is attained. In

watermelon, however, the cells are so constructed as to undergo far more expansion than this. Some small types, on the other hand, cease to enlarge at relatively small sizes. Whether these differences are due to different amounts of some substance which affects the extensibility of the wall, as auxin has been supposed to do, is a matter for investigation. There is some evidence that a more important factor in limiting the amount of expansion is the deposition of the secondary cell wall, which soon becomes so strong that further enlargement is impossible. The fact that it is commonly those types (notably the small-fruited forms) with the least amount of cell expansion which become the most hard and woody at maturity, and the ones with relatively great cell expansion which tend to remain soft (like the pumpkins and watermelons) seems to indicate the importance of secondary wall formation in limiting growth. Quite apart from either of these factors, the concentration of cell sap and the general expansive power of the cell may possibly be of much importance in determining how far it will enlarge.

The rather wide differences in amount of cell expansion, together with the variations in cell size at the last division, result in a good deal of variation in cell size at maturity even in closely related races, larger cells generally being associated with larger fruits. This conclusion is at variance with that of Sachs and Amelung. Indeed, with so many factors operating in determining cell and fruit size, it would be remarkable if cell size always ultimately reached the same value and if fruit size differences were due entirely to differences in cell number.

The relations between cell size and organ size here described apparently hold in other parts of the plant, such as the petiole (Sinnott, 1930). In the fundamental tissues of most axial organs there is a descending gradient in cell size from within outward which would probably show the same developmental history as that described here for the fruit. Analysis is more difficult in such organs, however, since the duration of growth is relatively short.

We may conclude that the relation of cell size to organ size is a complex one and that it is intimately involved with the whole developmental process. It cannot be solved merely by comparing the sizes of the two structures at maturity, as has usually been the procedure. A careful descriptive analysis of the whole course of development in quantitative terms is first necessary, not only to discover how the relations which are found at maturity have arisen but to distinguish the various processes which are concerned in growth and differentiation. This will open the way for an experimental study of each of these processes and thus for an ultimate attack on the whole problem of development.

### SUMMARY

In twelve races of cucurbits belonging to four genera, cell diameter was measured in a series of tissues from the central region of the ovary to the outside and at many stages during the developmental period from very small ovary primordia to mature fruit.

In each tissue growth takes place at first chiefly by cell multiplication, though cell size slowly increases also. After a specific cell size is reached. division ceases, and all further growth of this tissue is by cell expansion.

The innermost tissues, as compared with the successively outer ones, show (a) more rapid increase in cell size during the period of cell division, (b) earlier cessation of division, and (c) greater cell size at the time when division ceases.

In Cucurbita Pepo, large-fruited races as compared with small-fruited ones typically show (a) no difference in cell size during early development; (b) a more extended period of cell division, due to less rapid increase in cell size, greater cell size at the time of last division, and a greater interval between the cessation of division in successive tissues; and (c) greater cell expansion after cell division ceases.

Differences in fruit size are therefore usually due to differences in both cell number and cell size, though either factor may alone be responsible in certain cases.

Specific differences in development between Cucurbita and the three other genera are described.

Possible factors responsible for the differences in cell division and in cell expansion between the various tissues and in the various races are discussed.

The problem of the size relation of cell to organ is part of the more general problem of the factors determining growth and differentiation. The first step in the solution of this problem is a thorough descriptive analysis in quantitative terms.

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### AUXIN DISTRIBUTION IN FRUITS AND ITS SIGNIFICANCE IN FRUIT DEVELOPMENT 1

Felix G. Gustafson

GROWTH SUBSTANCES or auxins, as they will be called in this paper (Went and Thimann, 1937), are now generally considered to be factors in vegetative growth of plants. Recent investigations on artificial parthenocarpy produced by chemicals (Gustafson, 1936; Hagemann, 1937; Gardner and Marth, 1937) have shown that auxin under artificial conditions can also cause fruit development without fertilization. This coupled with the fact that auxin has been shown to be widely distributed in the vegetative parts of plants leads one to assume that auxin is also concerned with normal fruit growth. Gustafson (1939) has proposed the theory that a high auxin content in the ovary in the flower bud stage is responsible for natural parthenocarpy, which is found

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in many plants. In this paper it was shown that the auxin content is actually much higher in the ovaries of those plants that produce fruits parthenocarpically than in similar varieties requiring fertilization for fruit production.

Before it is possible to proceed very far with this matter, it is first necessary to investigate the auxin distribution in fruits. In 1936 Dollfus published some experiments which tended to show that auxin is produced in the developing seeds. He used the diffusion method. In these experiments he found that a substance diffused from the ovules into the agar which caused Avena coleoptiles to curve, but no such substance diffused out of the outer part of the ovary. Meyer (1936) has also found auxin in fruits. He extracted the auxin with alcohol in a reflux condenser for two hours and mixed the extract from 10 grams of fresh material with 1 gram of lanolin, which was applied to one side of intact Avena coleoptiles. While this method is perhaps not so good as the method in which agar containing the auxin is applied to decapitated etiolated Avena coleoptiles, nevertheless it is fairly accurate. In these experiments Meyer used a number of species of plants, and in the fruits of all these species he was able to demonstrate the presence of auxin. He did not do much with the distribution within the fruits, but in the few determinations he made, seed material gave a greater Avena coleoptile curvature than the other parts of the fruit.

The present work was undertaken to investigate further the auxin distribution in fruits. Fruits from many plants, in different stages of development and

during different seasons, were used.

PROCEDURE.—The work here reported was done at the William G. Kerckhoff Laboratories at the California Institute of Technology. Part of the experimental material was grown especially either in the greenhouse or in the field, while some of it, as the Yucca Whipplei, Agave Brundigii, Pittosporum undulatum, and Clivia sp., was obtained from the Huntington Gardens at San Marino. The auxin content and distribution was determined by the Avena method, the technique of which has been discussed in detail by Went and Thimann (1937).

Auxin was extracted with freshly distilled ether according to Van Overbeek's (1938) modification of other methods. The dried residue was thoroughly mixed with a known amount (usually .4 to .8 cc.) of 1.5 per cent agar and after standing 90 minutes was poured into a nickel plated brass mold 1.5 imes 8.0 imes10.5 mm. in size. After the agar had been cut level with the surface of the mold, the large block was cut into 12 equal pieces. The material was now ready for the test. Great care was taken to have freshly prepared extracts. Usually the extract was prepared the same day that it was used, but if the extract could not be used the same day that it was made, it was always stored in a refrigerator in dried condition. Fleshy material was repeatedly extracted until the Avena test plants showed no curvatures when treated with the agar-extract. In some experiments such material as tomato and squash was first frozen with liquid air then ground in a mortar and the ether added. The extraction was always carried out in the dark and usually, though not always, at a low temperature.

Control experiments in which Avena plants were treated with a known concentration of indole acetic acid (usually 21.5 gamma per liter) were run with every experiment. (This concentration ordinarily gave a curvature of about 10-12°.) By this means the sensitivity of the test plants was determined for each experiment, which was very important, because there was considerable variation day by day, and the auxin concentration in a plant can be calculated in terms of indole acetic acid (see Van Overbeek) rather than in terms of some of the many units which have been employed by different investigators. This procedure has the advantage that the activity of the growth substance or substances is recorded in terms of a known and easily obtainable substance, indole acetic acid. One does not know what the substance is nor is its concentration known, but it is known that its activity on a decapitated Avena coleoptile is equal to a known concentration of indole acetic acid.

A few experiments were performed in which the plant material was placed on the agar blocks as in Went's original experiments. In most instances such blocks when placed on Avena coleoptiles caused no curvatures. It had been hoped that this method could be used, but as so few plants gave any results, it was abandoned for the extraction method. This brings up a question which is in the minds of manynamely: Is extracted auxin the same as that which diffuses into the agar block, and if so, is the concentration in the plants that give negative results so low that only by using a large quantity of material can a test be obtained; or is the auxin which is obtained by extraction released from a combined form, with low diffusibility, by the extraction treatment? Those experiments in which, by daily extraction, active material continued to be obtained for a period of 10 days certainly suggest an affirmative answer to the latter alternative.

In these experiments the fruits were separated into an interior and exterior part, or, as in the tomato and summer squash, further subdivisions were made. When a fruit was divided into only two parts, the interior part included the central axis, the placentae, the ovules and all the cells in between and immediately surrounding the ovules, and the exterior part usually included only the ovary wall, as in the peppers, tomatoes, beans, and Yucca. In the second experiment with peppers, listed in table 1, the beans and Yucca, only the ovules and seeds were included in the interior part. The extraction method varied considerably in these experiments. All material was cut into rather small pieces to begin with, but in some experiments these pieces were frozen with liquid air, and while they were in the frozen condition, attempts at maceration were made. These differences in treatment of the material should in no way influence the results, as all material experimented with at any one time was always treated in the same way, and extractions were continued until no further reaction was obtained with the Avena coleoptiles.

The investigation was divided into two parts. Part one dealt with the auxin distribution in nonparthenocarpic fruits, and the second part with a comparison of auxin concentration in artificial parthenocarpic and non-parthenocarpic fruits. In the second part the same plants were used, and the extractions were of course made at the same time.

RESULTS AND DISCUSSION.—Distribution of auxin within non-parthenocarpic fruits.-In determining the auxin distribution within fruits, a number of different plants were used, and the experiments extended over a period of five months from March to August. The differences in auxin contents that have been noted in different experiments with the same species of plants are probably partly due to seasonal differences and partly to difference in the vigor of the plants. The tomatoes in table 1, the first experiment with peppers in table 1, and all the peppers in table 5 were grown in the greenhouse during the winter or early summer, and the plants

Table 1. Auxin distribution within fruits. The interior includes only ovules or seeds in Yucca, beans, and the second experiment with peppers; otherwise it includes the central axis, the placentae, ovules, and all cells in between or closely associated with them. Figures denote auxin concentration in terms of indole acetic acid equivalents in gammas per kilogram green weight, except in the diffusion experiment with Pittosporum. The second experiment with peppers, the experiments with cucumbers, crookneck summer squashes, and beans were made during the summer, and the others during the winter or spring.

		Weight of mate- rial, in		Weight of mate rial, in
Plant	Interior	grams	Outside	grams
Pepper (first experi-				
ment)	0.16	31.7	0.07	156.2
Pepper (second ex-				
periment)	61.90	3.95	2.29	64.0
Tomato	0.06	91.40	0.02	276.60
Agave Brundigii	0.27	31.0	0.06	85.0
Cucumber	5.06	25.7	1.99	81.0
Crookneck summer squash				
Buds	14.09	7.32	7.52	12.66
Flowers	7.30	16.42	1.21	21.50
Young fruits	0.58	36.55	0.00	124.15
Kentucky wonder				
bean				
Pods 7-10 cm	108.80	2.19	3.24	64.1
Pods 12-14 cm	20.89	8.55	1.41	152.5
Pods about 19 cm.	7.00	33.66	0.46	73.1
Yucca Whipplei				
Fruits $1.8\times2.7$ cm.	0.34	19.55	0.12	42.05
Fruits 2.3×3.4 cm.	0.67	22.28	0.13	38.04
Fruits $2.5 \times 3.5$ cm.	0.23	26.74	0.12	40.58
Pittosporum undula-				
tum (diffusion) 24				
Avena coleoptiles				
used	4.6°		0.00	

were not particularly vigorous, while the second experiment with peppers employed fruits grown in the field, where the plants were much more vigorous. The fruits were also larger. Even though there was considerable difference between different lots of fruits, the relative concentrations within the fruits were much the same in all experiments with the same fruit. The auxin concentration was much lower in the winter and spring than in the summer.

From the information gained in these experiments Yucca in which only seeds or ovules were used. In it seems as if the seeds or developing seeds must be centers of auxin production. This is particularly indicated by the experiments with peppers, beans, and the other fruits other cells adjoining the seeds were used, and the results are not so positive for them. To clear up this point, crookneck summer squash and tomatoes were used in several experiments in which the seeds were separated from the rest of the fruit, which was further subdivided.

Table 2. Auxin distribution in fruits of tomato and crookneck summer squash. Auxin concentration is denoted in terms of indole acetic acid equivalent, and the figures denote gammas per kilogram of fresh material. These experiments were made during the summer.

Plant and condition of fruit or ovules	Tissue around ovules	Ovary wall	Neck of squash without seeds
Squash, yellow, 40			
cm. long, wt.			
772.2 g 1.31	3.38	0.48	0.12
Tomato, green, 4-5			
cm 10.60	2.15	0.36	•

In two experiments tomato fruits were separated into five parts and these parts extracted separately. Included in one of these experiments were parthenocarpic fruits produced by phenylacetic acid. Phenylacetic acid is known to give practically no Avena test, and therefore fruits were produced by its stimulation to determine whether these parthenocarpic fruits make or at least contain auxin. Table 3 gives the result of these experiments.

These experiments show that the ovules or young seeds are high in auxin content and that the other parts of the fruits contain much less auxin. Whether this auxin is produced in the ovules or diffuses into them from the leaves, we have no way of knowing from these experiments. Nevertheless, the results as a whole indicate that growth (however initiated) sets up a concentration gradient with the highest concentration in the ovules, when present, otherwise in the central axis and partition region and with a low concentration in the ovary wall.

Table 3. Auxin distribution in tomato fruits, produced by pollination and parthenocarpically by by phenylacetic acid. The fruits were green and between 4.5 and 6.0 cm. in diameter. Auxin concentration is denoted in terms of indole acetic acid equivalent, and the figures indicate gammas per kilogram of fresh material. These experiments were made during the summer.

Type of fruit Seed	Jelly around seeds or ovules	Placentae	Central axis and partitions	Pericarp
Normal (Exp. 57) 15.33	6.57	2.44	2.27	1.27
Normal (Exp. 65) 30.05	0.45	2.47	2.69	0.63
Parthenocarpic (Exp. 65)	1.25	0.49	6.42	0.81

Comparison of auxin content in parthenocarpic and normal fruits.—In the preceding section it has been shown that the ovules or seeds of fruits have a high auxin content. In order to answer some of the questions which have been raised concerning the growth of fruits, it is necessary to know whether or not there is auxin production or accumulation in fruits when no seeds are produced.

It is a well-known fact that in certain interspecific crosses or even in intergeneric crosses, the ovary may develop into a fruit without any seeds. According to Yasuda (1935) the pollen tubes bring into the ovary something which stimulates growth. This is undoubtedly auxin (Gustafson, 1937). The auxin brought into the ovary is certainly not sufficient to cause continued growth, and the query has been made as to where this additional growth substance

Table 4. Comparison between auxin concentration in normal and parthenocarpic tomato fruits. Auxin concentration is denoted in terms of indole acetic acid equivalent, and the figures indicate gammas per kilogram fresh weight.

Pol	linated	Parthenocarpic	
Condition of fruit Perican	p Interior	Pericarp	Interior
Green, 3.0 ×			
4.0 cm. diam 4.00	27.03	0.40	0.72
Green, 4.6 ×			
6.5 cm 2.57	7.53	0.73	2.06

comes from. Another variation of the above question has been concerned with the growth of normal parthenocarpic fruits as oranges, lemons, and grapes. Where does the auxin come from that causes them to grow? There is probably no pollination and no development of seeds. In artificial parthenocarpy produced by chemicals, interest has centered around the continuation or non-continuation of growth in a fruit after the initial stimulation. Gustafson (1937, 1938a, 1938b) has reported a number of instances in which the initial growth stopped very soon, whereas at other times a fruit continued to grow until half size and then stopped; in still other fruits growth continued until maturity. What is the cause of this difference in behavior? Is the auxin supplied to the ovary in the form of paste or spray causing all of the growth during the enlargement of the fruit, or does the developing fruit itself supply a part? Gardner and Marth (1937) found that four sprayings produced a larger percentage of setting than

a single spraying. It is not clear whether this means that a greater percentage of flowers on a branch produced fruits or whether a larger number of treated flowers produced fruits. From the sentence following their statement that several treatments are more efficacious than a single one, it seems that they have reference to the whole branch. "In the practical use of these compounds in producing fruit on holly, successive spraying would be necessary in that the blossoms are not all open at one time." Several injections of auxin into ovaries and fruits of tobacco produced no more growth than a single injection, according to Gustafson (1938a). If Gardner and Marth's statement has reference to a cluster of holly flowers as a whole, it would seem that the added auxin merely initiates the growth and that the ovary either produces or obtains from the leaves enough for its continued growth and that if this source is not sufficient for the needs, the fruit stops growing.

To obtain information on this point, tomato fruits were produced parthenocarpically by means of phenylacetic acid. Phenylacetic acid, as mentioned before, has only a very slight influence on the Avena test. The auxin determination was made in the usual way. Table 3 shows one of these experiments, and in table 4 are found two more determinations on such fruits.

Parthenocarpic fruits produced by naphthalene acetic and indole butyric acids were also used, and table 5 gives the results for peppers. These fruits were grown in the greenhouse, during the spring and early summer.

According to Went and Thimann (1937), indole butyric and naphthalene acetic acids produce slight curvatures in Avena coleoptiles so that we have no information about the naturally occurring auxin in these experiments, but they definitely demonstrate that there are growth promoting substances in the growing fruits to which the Avena is sensitive; whether these are the substances added at the time of flowering or natural growth hormones, we do not know. The concentration of this active substance is lower than in normal fruits, however.

In the experiments with fruits produced by phenylacetic acid there is no doubt that parthenocarpic tomatoes contain naturally occurring auxin. If auxin occurs in tomatoes, one would expect it to be present also in other fruits. Whether this auxin is produced in the fruit or conducted into it from other

Table 5. Comparison between auxin content in normal and parthenocarpic peppers. Amount of material used is denoted in grams inside the brackets. Auxin concentration is denoted in terms of indole acetic acid equivalents, and the figures indicate gammas per kilogram of fresh material.

Normal fruits	Parthenocarpic whole fruit		
Seeds and placentae	Pericarp	Naphthalene ac.	Indole butyric
0.16 (31.7)	0.07 (156.2)	0.07 (114.0)	
0.53 (15.0) 0.80 (16.6)	0.1 (100.0)	0.25 (49.5)	0.18 (51.0)

sources, we do not know, and it need be of no concern. That it is there is the main point.

It then seems very likely that in some fruits there is a quantity of auxin sufficient for the needs of continued growth, after growth has once been initiated, even though no seeds are produced, while in others the amount is too low for the needs of a continued growth, and development ceases after some time. In varieties where mature parthenocarpic fruits have never been obtained, it is likely that the naturally occurring auxin content is always too low to sustain growth after the artificial supply has been depleted, and the only way such fruits can be made to grow and mature is by continuously supplying them somehow with auxin as do the seeds in non-parthenocarpic fruits, but we have not yet discovered a method of successfully doing this.

In normal fruits in which pollination and fertilization are necessary we can consider it a reasonable hypothesis that the initiation of growth of the ovary into a fruit results from the auxin brought into it by the pollen tubes. If this is true, then one might expect that right after pollination the auxin content should be higher than just before. Meyer reports that just after fertilization, which might have reference to pollination, the auxin concentration in the female flowers of Cucurbita Pepo, Cucumis sativus, and Helianthus annuus was much higher than just before fertilization, and he also makes the general statement that there is less growth hormone in female flowers before fertilization than afterwards. In the present investigation there is little if any evidence that there is more auxin after pollination than before. In fact, it would be very difficult to get such information when much material is needed for extraction. Even if it could not actually be shown that the auxin content was higher after pollination, that is no serious criticism of the hypothesis, because even a little auxin added in the right place, as the ovules, would be sufficient to cause them to grow and set up auxin centers from which auxin might diffuse into the ovary. In a preceding paper (Gustafson, 1939), it was shown that in two-week-old Paper rind and Valencia seeded oranges the auxin content had increased very much over that in the flower stage. After the embryo has commenced growing, auxin in considerable quantity is present in the seed. This auxin undoubtedly diffuses into other parts of the ovary, where growth takes place. Even if seeds are not produced, there is auxin present in the ovary.

Some may hesitate to accept the idea of auxin playing such an important rôle in the growth of fruits. Let these consider that fruits have artificially been started and caused to develop by the addition of auxin. If auxin is able to do this when added from the outside, is there any reason why it should not play an important part under normal conditions? Furthermore, let it also be remembered that the mere pollination without fertilization does sometimes cause fruits to develop, and auxin has been extracted from pollen (Laibach, 1932; Thimann, 1934), and Gustafson (1937) extracted a substance which, when placed on the pistil of a flower, caused it to develop into a fruit. All these experiments show that there is auxin in the pollen and that it may cause growth of fruits.

In spite of these statements it is to be understood that auxin is not considered to be the only factor involved in fruit growth. In a recent publication Went (1938) considers that auxin acts in such a way as to cause other substances which he calls calines to move to the part of the plant where growth takes place. Thus he considers that auxin causes the caulocaline to move to the apex of a stem where it functions in the growth of the stem. The auxin may then be thought of as a master reagent causing other substances to produce growth of one sort or another. If this idea is applied to the growth of fruits, it becomes obvious that auxin causes some other substance or substances to move into the ovary of a flower and there cause growth to take place. It is not necessary at the present time to have a name for such a hypothetical substance, but by analogy it

### SUMMARY

would be carpocaline, if different from other forma-

The auxin content of ovules and developing seeds is much greater than that of other parts of fruits, and it is considered possible, even though not yet proved, that they produce auxin.

Auxin other than that added artificially is found in fruits that have been produced by treating the flower by phenylacetic acid.

The probable rôle of auxin in the growth of parthenocarpic and non-parthenocarpic fruit is discussed.

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tive substances.

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### OBSERVATIONS ON THE GENUS PSEUDOLPIDIUM 1

### D. A. McLarty

Following the early observations of Nägeli (1846), several workers, including Cienkowski (1855), Braun (1855), and Pringsheim (1860), reported the presence of various bodies in swollen filaments of water molds; but little intense work was done on them until Cornu (1872) published the results of his studies. He recognized these bodies to be parasites and described several species, in three of which he observed spiny, thick walled resting spores with one or more smooth or slightly echinulated, thin-walled, empty cells attached to them. Cornu considered these spores to have arisen from a fusion of a smaller male thallus with a larger female one and interpreted the empty cells as the antheridia. Using this sexual spore character as the diagnostic feature, Cornu thus established the genus Olpidiopsis for these small, intramatrical parasites.

Later Fischer (1880), studying what he thought to be Cornu's type species O. Saprolegniae, failed to find resting spores with attached antheridial cells and came to doubt Cornu's earlier observations. In 1882 he rejected the "adjacent cell character" and restricted the genus Olpidiopsis to forms which possessed asexual resting spores. Subsequent studies, however, convinced Fischer that his earlier work was incorrect, and in 1892 he confirmed the observations of Cornu and reinstated the genus Olpidiopsis to its original status. At the same time, he established the genus Pseudolpidium to include Olpidiopsis-like species which produce resting spores without adjacent cells. Of the six species which Fischer recognized, only two are tenable, inasmuch as he did not observe the resting spores of four of them. In P. Saprolegniae he described smooth, flatspherical to ellipsoidal zoosporangia (fig. 5, 6) and spiny resting spores of similar size and shape (fig. 9). In P. fusiforme Fischer described smooth, long ellipsoidal to cylindrical zoosporangia associated with similar long, spiny resting spores (fig. 2).

Butler (1907), studying various other species of Pseudolpidium, has not supported Fischer with ref-

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The writer wishes to express appreciation and thanks to Prof. H. B. Berdan, of the University of Western Ontario, for the culture from which this study was made, and to Prof. J. S. Karling, under whose direction this investigation has been carried on, for his help and criticism.

erence to this striking similarity between the zoosporangia and resting-spores but in all cases describes and figures spherical, definitely thick-walled, spiny resting spores which differ from the *Olpidiopsis* type of spore only in the absence of the adjacent cells.

In swollen filaments of Achlya secured in November, 1937, conspicuous, cylindrical, smooth sporangia (fig. 10) were observed together with long, spiny sporangia (fig. 11) as described by Fischer for P. fusiforme. In the same culture smooth, ellipsoidal zoosporangia (fig. 14) were present as well as spiny sporangia of the same size and shape (fig. 15), which have been described by Fischer for P. Saprolegniae. Dependent apparently upon conditions of growth, zoosporangia were found to vary greatly in size and shape, and in any culture a complete series from spherical to cylindrical could be observed. Spiny sporangia showed similar variation in size and shape, and the degree of spininess was found to vary from slight echinulations to heavy bristles. These bristly bodies showed no tendency to rest but liberated zoospores readily, thereby proving themselves to be nothing more than zoosporangia which,

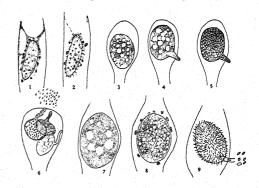


Fig. 1-9 were copied from plates given by Fischer and Pringsheim.—Fig. 1-2. Stages in the development of the resting-spore of Pseudolpidium fusiforme (127×, after Fischer).—Fig. 3-5. Stages in the development of the zoosporangium of Pseudolpidium Saprolegniae (127×, after Fischer).—Fig. 6. Zoosporangia of Pseudolpidium Saprolegniae (87×, after Pringsheim).—Fig. 7-9. Stages in the development of the resting-spore of Pseudolpidium Saprolegniae (117×, after Fischer).

probably in response to some condition of the host protoplasm, become covered to a greater or lesser degree with bristles. For the sake of clarity it seems advisable to refer to these as bristles in contrast to the heavy, broad-based spines found on the wall of true resting spores. Spherical, thick-walled, asexual resting spores (fig. 17), of the type described by Butler for various species of *Pseudolpidium*, were present as well as spores of the *Olpidiopsis* type (fig. 16).

Monospore cultures were established from zoospores isolated from all types of sporangia present in the infected Achlya. Regardless of the type of sporangium from which the spore was taken, however, the monospore cultures were all precisely the same. All the sporangial types present in the original culture were represented in the monospore cultures along with resting spores formed with or without adjacent cells. In consecutive culture generations the ratio of sexual to asexual spores, as indicated by the presence or absence of adjacent cells, varied considerably, although the asexual spores remained always the more numerous.

Inasmuch as the bodies which Fischer described as resting spores are merely zoosporangia bearing bristles, Fischer's description of the genus Pseudolpidium must be revised to include zoosporangia of this type as well as smooth walled sporangia. In view of Butler's work, it seems necessary to maintain the genus Pseudolpidium to include his asexual species. Here again, however, the genus must be revised in relation to the spherical, heavy-walled resting spores. Moreover, monospore cultures indicate that the structures which Fischer described as zoosporangia and resting spores of two species are simply modifications of the zoosporangium of a single organism.

Sexuality, in this form at least, is not well established and definite, but appears to be rather a potentiality the expression of which may possibly be associated with or dependent upon certain obscure cultural conditions. In view of this evidence the nature of the resting spore is not a suitable character upon which to base generic distinctions. Cytological investigations now in progress may serve to explain the nature of sex in these two closely related genera. For the present we may consider this form as a species of *Pseudolpidium* which is predominately asexual but which, under certain conditions, may dis-

play some sexuality. Careful study of the species of Olpidiopsis and Pseudolpidium may reveal that there are many such transitional forms or that all sexuality in this connection is relative.

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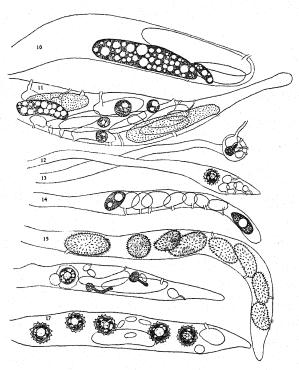


Fig. 10-17 were drawn from living material with the aid of a Zeiss prism. Magnification  $96 \times .$ —Fig. 10. A swollen filament of Achlya containing four cylindrical, smooth, thin-walled zoosporangia.—Fig. 11. A heavily infected Achlya filament showing two bristly, cylindrical zoosporangia associated with thin-walled, smoth, spherical and fusiform zoosporangia.—Fig. 12-13. Dwarfed Achlya filaments bearing several small, smooth zoosporangia and a single asexual resting spore.—Fig. 14. Several flat-spherical, smooth zoosporangia contained in a swollen Achlya filament.—Fig. 15. A swelling containing flat-spherical bristly zoosporangia.—Fig. 16. A single immature sexual resting spore in a swelling with several zoosporangia.—Fig. 17. A swollen filament containing five asexual resting spores.

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### MEDULLOSA DISTELICA, A NEW SPECIES OF THE ANGLICA GROUP OF MEDULLOSA <sup>1</sup>

James M. Schopf

MEDULLOSA DISTELICA is the third species of this genus to be discovered in America. Some years ago Thiessen (1920a, 1920b) illustrated a pyritized tristelar Medullosa in connection with his studies of bituminous coal. This form was identified as Medullosa anglica, but after reexamination of Thiessen's specimen, it seems best to class it as a distinct variety. The origin of Thiessen's specimen is unknown beyond the fact that it had been collected on one of the earlier reconnaissance trips in the western coal fields of this country.2 Medullosa noei, the second species, was recently described by Steidtmann (1937). It is from coal balls found in a thin locally mined coal a few miles east of the town of Calhoun, Richland County, southeastern Illinois. The limestone above the coal at this locality has been described (as LaSalle) by Newton and Weller (1937) and is placed at about the middle of the Mcleansboro formation. This formation is considered roughly equivalent to the Conemaugh of eastern United States. In Richland County the Herrin (No. 6) coal at the base of the McLeansboro is about 800 feet below the Calhoun coal.3 The third species, here described as Medullosa distelica, is from coal balls out of the upper part of the Herrin (No. 6) coal bed (Cady, 1936) in the mine of the Clarkson Coal and Mining Co. at Nashville, Washington County, Illinois. The Nashville coal balls were discovered in April, 1935, by G. H. Cady, L. C. McCabe, and C. G. Ball of the Illinois Survey. These calcareous concretions have provided a variety of fossil plant material representative of that which contributed to the formation of the upper part of this coal bed. New plants, such as the one treated in this paper, will be described from time to time independent of studies on the botanical constitution of coal, since such fossils have special botanical and stratigraphic significance.

Medullosa distelica sp. nov.—Stem oval, of medium size, measuring  $ca.8 \times 4$  cm. when slightly compressed. Two highly asymmetric protostelic cylinders each  $ca.7 \times 18$  mm. in diameter with mixed "pith" occur within the bandlike internal periderm. Secondary wood strongly developed on

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Geological Survey, Urbana.

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<sup>2</sup> Reinhardt Thiessen, personal communication to the

author, October, 1936.

<sup>3</sup> Records of the Division of Subsurface Geology, Illinois State Geological Survey.

each stele toward the center of stem, much less developed on external sides of each stele; little or no secondary xylem associated with outgoing leaf traces. Internal periderm 11/2-3 mm. broad and originally probably continuous around the stelar tissues. The periderm band is not broken where the outgoing leaf traces pass through. Three extremely decurrent leaf bases occupy the outer 1-2 cm. of stem. two of which are demarcated internally by a sclerenchyma zone. Internodes long. sclerotic rind is of Myeloxylon landroitii type, 2-4 mm. thick, with its internal boundary not strictly definable. Secretory ducts enclosing "resin rodlets" numerous and commonly associated with sclerotic strands. Leaf trace bundles are chiefly of the typical centripetal collateral type; they are a little more massive when entering the leaf base but soon bifurcate to assume their typical configuration.

The holotype was derived from an approximately spherical coal ball. The stem was situated somewhat excentrically within the nodule and exposed in slightly oblique section by the first exploratory cut. About 5 inches of the stem was available for

study

Figures 1 and 2, plate 1, are taken from opposite extremities about 4 inches apart, where in each case the specimen is fairly complete. Figure 1a is a tracing taken from the photograph in 1b. No leaf traces are indicated in this or other drawings. The stelar structures, similar to those shown in plate 1, figure 1a, have been drawn at successive levels approximately an inch apart and are shown in their normal succession in text figure 1c, d, and e; figure 2, plate 1, is thus subjacent to text figure 1e.

The phyllotaxy merits no special comment at this time except to note that the same leaf bases are present throughout, and thus a long internode is indicated. Internal sclerenchyma setting off two of the leaf bases is present, making it more like Medullosa centrofilis (De Fraine, 1914) than M.

anglica in this respect.

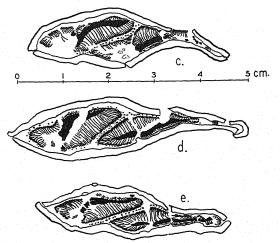
One of the distinct features of this species is the extreme asymmetry of the xylem cylinders. By asymmetry is meant deviation (in this case bilateral) from the radial development common to most steles of vascular plants. In Medullosa distelica asymmetric stelar growth is most prominently displayed by the secondary wood which is narrow on the external side of the individual stele and much thicker internally. Medullosa anglica (Scott, 1899) shows similar asymmetry of the steles but in a lesser degree; thus far this feature has attracted little attention. In M. anglica the asymmetry of growth might be explained, at least in part, as due to depletion of the peripheral wood by outgoing leaf traces. Since the leaf traces are considerably less massive in M. distelica than in M. anglica, they are probably not directly responsible for the more extreme thinning of external secondary wood in the Nashville specimen. The contrasting development of internal and external secondary wood  $(x^2)$  at the end of the steles is shown in figure 3, plate 2. Flattening of the two steles in M. distelica, as shown in text figure 1c, d, and e, may be due to the mechanical pressure developed by growth of one stele against the other.

The internal zone of secondary wood is relatively thick, up to about 5 mm. The normal tracheids are large in transverse section (150  $\mu$  or more in diameter) and slightly broader in a radial They are aligned in rows of one, two, direction. or three cells' width between rays. The rows more than one cell in width generally show the alternating arrangement of tracheids characteristic of Medullosa wood (fig. 3). This is probably indicative of cambial activity somewhat different from that found in most modern plants. The tracheids are covered with characteristic multiseriate pits on their radial and obliquely tangential walls (fig. 7); few pits are present on the squarely tangential faces. The rays are numerous (4 or 5 per mm. as measured tangentially) and narrow. They appear as parenchymatous continuations from the mixed "pith," generally several cells wide when first entering the secondary tissue, but for the most part they soon diminish to one or two cells in thickness. In tangential section the rays are fusiform and, although their height is difficult to determine from this specimen, some apparently extend vertically for as much as a centimeter. The last formed cells of each xylem row decrease in size approaching the margins, as shown in figure 3, until the last formed elements in this area are less than  $\frac{1}{4}$  the size of those forming the normal secondary wood. In this last formed (incompletely differentiated?) part of the secondary cylinder the rays are again a little wider.

Neither phloem nor pericycle is preserved and several Stigmarian rootlets have invaded the area between xylem and periderm. The space between the two steles contains some dark disintegrated material and a row of black "resin rodlets" similar in character to those of the petioles, except that they are considerably smaller. These rodlets are indicated by the rows of dots between the steles in text figure 1. Little except the presence of these secretory elements can be determined of the tissues immediately outside the secondary wood.

The primary wood (mixed "pith") is poorly preserved except for clusters of large primary tracheids (plate 2, fig. 3,  $x^1$ ). In some places the intervening parenchyma is present, but in many places Stigmarian rootlets have also invaded this region. Thus far position of the protoxylem has not been assuredly identified. However, since the internal cells of the secondary xylem are well preserved in part and show no protoxylem directly adjacent, it seems likely that M. distelica developed mesarch protoxylem.

The species is characterized by development of a very narrow zone of secondary wood on the external side of each stele as shown in plate 2, figure 3 (outer  $x^2$ ). In places the primary wood seems to have been in direct contact with the pericycle; lat-



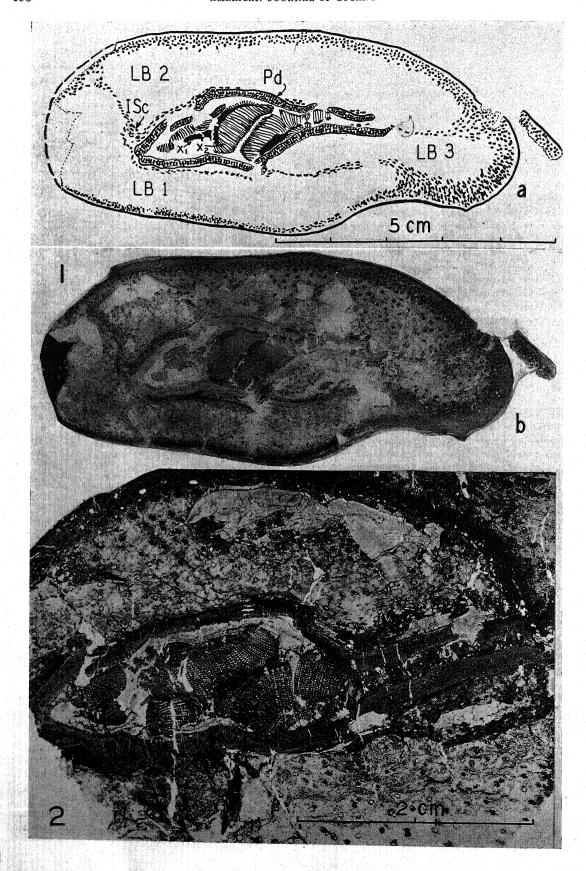
Text fig. 1. Medullosa distelica. Diagrammatic tracings of the vascular tissues and internal periderm in sections spaced about 1 inch apart. Primary xylem, black; secondary xylem, lined. Sections are in their natural order, c below the section shown in plate 1, fig. 1; e above the section shown in plate 1, fig. 2. The tissues are considerably broken in certain areas due to intrusion of foreign roots and small veins of secondary calcite. c from 49C (B2), d from 49C (T1), e from 49B1a (B1), Illinois State Geological Survey collection.

erally from these spots the secondary tracheids increase in number, particularly toward the thick inner wood.

Outside the steles there are a few isolated bundles of primary tracheids which apparently went to supply the petioles. Although the structure of these is not very satisfactorily shown, it seems certain that few or no secondary tracheids accompanied the leaf traces away from the steles. This is a character in considerable contrast to Medullosa anglica where radially oriented secondary xylem continues around the outgoing leaf trace for several centimeters.

In a few instances somewhat dislocated leaf trace bundles were observed completely surrounded by the periderm or secondary cortex (plate 3, fig. 13, VB). The bundles, nevertheless, may have functioned normally. Although De Fraine (1914) and more recently Steidtmann<sup>4</sup> have pointed out that the internal periderm was probably not an impervious suberized cork layer (morphologically it resembles corky tissue) (fig. 13, Pd), the bundles traversing it constitute the most likely channels for conduction. In Medullosa anglica the leaf traces accompanied by secondary wood were apparently too large to be completely enveloped by the narrow periderm, and hence they form a gap in the periderm sheath where

4 Unpublished manuscript.



they pass through it to the leaf base. Although periderm surrounds the smaller vascular traces for an interval in M. distelica, there is no reason to suppose that the course of conduction was different from that in M. anglica.

Since Medullosan material is not abundant in the Nashville coal balls, the Myeloxylon petioles found there most probably all belong to Medullosa distelica. Traverses measured across a representative series of these concretions show that various tissues of Medullosan origin are present in not more than  $2\frac{1}{2}$  per cent of the area. In the other species of Medullosa the sclerenchymatous rind (or hypoderm) of both stems and petioles has been sufficiently similar in character to indicate rather conclusively the affinity of associated but isolated petioles. If the Nashville stem and associated but unattached petioles belong together, it is apparent that the rind varies somewhat more from stem to petiole on this plant than is reported for other species. (Compare fig. 9 and 12, plate 3, with 1b and 2, plate 1.) In the leaf bases attached to the stem the sclerenchymatous strands are small and numerous, similar to those of Myeloxylon landroitii described by Renault (1876). The strands in isolated Nashville petioles on the other hand tend to be a little larger and more radially elongated. In some sections they seem more like the M. radiata type of Renault.

The sector of a petiole illustrated in plate 3, figure 9, shows the leaf trace bundles to good advantage. It seems likely that the part of the petiole illustrated here was basal and had been broken from a stem. It agrees with the complete petiolar bases attached to the type specimen in width, and one side of the rind was missing as though it had been broken from its decurrent attachment to the stem. Furthermore, the bundles are seen in stages of bifurcation just as in the type specimen. A double bundle is shown at the lower right in figure 9 (DB). The pairing of recently bifurcated bundles more centrally placed is also shown.

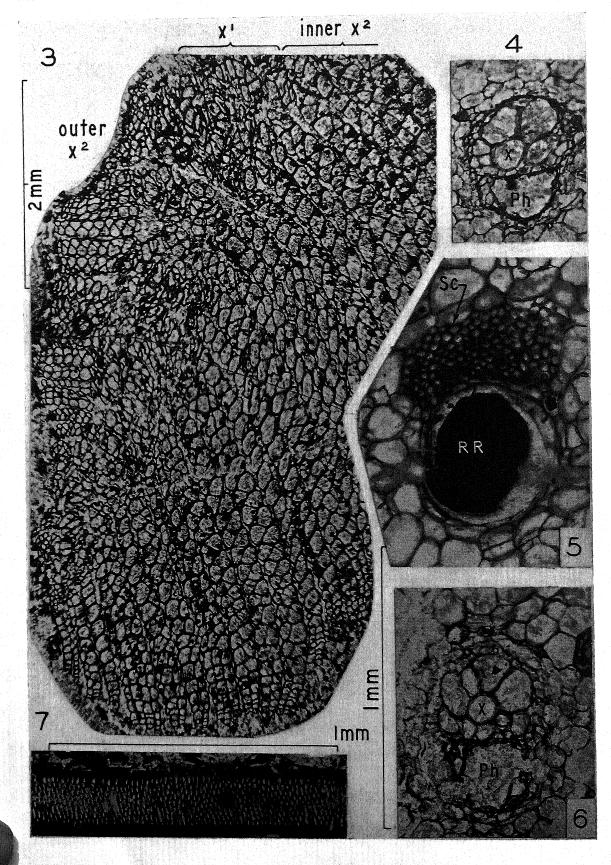
The collateral leaf trace bundles of M. distelica are shown in plate 2, figures 4 and 6. Cells of the phloem area (Ph) are almost entirely disintegrated, but the cavity which they formerly occupied is preserved without distortion. The xylem (x) consists of a few tracheids showing a gradation in size away from the small protoxylar tracheids placed next to the phloem. In M. distelica the bundle sheath is quite narrow, and the cell walls are not strongly thickened. The bundle sheath in figure 6 has become disintegrated, while the xylem tracheids are still adequately preserved. This may be indicative

of the comparative degree of lignification in the two

The secretory canals of the petioles are large and numerous (plate 3, fig. 9, RC). In the central part of the leaf base they generally lack any sclerotic accompaniment, but toward the sclerotic rind a strand of thick walled sclerenchyma is generally associated with them. The canals are also shown in plate 1, figure 2, and one accompanied by a small patch of sclerenchyma is shown at higher magnification on plate 2, figure 5. An epithelial layer is present. The contents of these canals have long been objects of interest to coal petrographers. Isolated from coal they are rod like in form and frequently preserve external imprints of the epithelial cells of the canals. Chemically they are very inert since they withstand drastic chemical oxidation with no observable change and are most like fusain in this respect. They have been called "resin rodlets," although it has long been realized that their resinous nature is inadequately proved. The name nevertheless serves to characterize them rather well, since they are essentially similar to resin in their mode of occurrence within the plant. A closer comparison of the secretory ducts might be drawn from the mucilage canals of certain Cycads, but it is hard to conceive of Cycadean mucilage assuming such a hard imperishable form. These resin rodlets are essentially opaque even in extremely thin sections, which of course makes their simple resinous nature more doubtful. The presence of these bodies in their typical form within well preserved Medullosan petioles and stems may be taken as evidence that the rodlets lost their fluid consistency very early, perhaps even before death of the plant. The process responsible for their present condition is one of considerable interest to students of coal as well as to paleobotanists, since it has bearing on the origin of fusain or mineral charcoal in coal. If the rodlets were even slightly volatile prior to coalification, the presence of characteristic and typical rodlets in both fusanized and unfusanized plant tissues would seem to rule out prehistoric forest fires as the agent responsible for this coal constituent. A volatile plant secretion presumable would have been distilled out by the heat. The rodlet (RR) shown in figure 5 has been slightly distorted by a cross fracture subsequent to the original mineralization.

Figure 12 shows two isolated petioles which may represent the main rachides further removed from their stem. One of these shows two secondary branches (b) being given off at about the the same

Plate 1. Fig. 1-2.—Fig. 1a. Medullosa distelica, transverse section of holotype. Interpretative tracing from photograph shown in figure 1b. LB 1, 2, and 3, leaf bases; ISc, internal sclerenchyma which delimits two of the leaf bases; Pd, internal periderm band; X<sub>1</sub>, primary xylem (mixed "pith"); X<sub>2</sub>, secondary xylem. Leaf trace bundles not indicated.—Fig. 1b. Medullosa distelica, transverse section of holotype, original of figure 1a. From 49D3b (T1). (This and other similar combinations of characters indicate the source of the material illustrated among the coal ball collections of the Illinois Survey. By use of this system the original location of the many nitrocellulose films and thin sections which were prepared may be rather accurately defined.)—Fig. 2. Medullosa distelica, transverse section of holotype near one extremity. The stele at the left is somewhat unusual and may be in process of division. From 49A (T4). Specimens in the Illinois State Geological Survey collections at Urbana.



level. It would seem from this and other similar occurrences that branching of the leaf axis was monopodial and opposite. No evidence of dichotomy has been observed, although Seward (1917, p. 87) has suggested that this method of branching was more common in *Medullosa* and other pteridosperms than in the true ferns. A secondary petiole is shown at intermediate magnification in plate 3, figure 11; the two bays of parenchyma invading the sclerotic rind at b and b', opposite one another and a little above the center, indicate the position of tertiary petiolets. An isolated petiole of this last order is shown in figure 10 at the same magnification as in figure 9.

Alethopteris-like leaflets are closely associated with tertiary petioles. The pinnules are 21/2-3 mm. broad, about half as large as those associated with M. noei. Between veinlets the tissues include the following: upper epidermis of small cells, hypodermis (usually much better preserved than the epidermis) of large cells with secretory contents above veinlets, and chlorenchyma of medium sized cells slightly elongated at right angles to the plane of the blade. A large unobstructed air space separates the lower epidermis (with cells somewhat larger than those of the upper epidermis) from the chlorenchymatous tissue. Chlorenchyma cells are one or two layers deep above the air space but more numerous around the border tissue of veinlets. The mid-vein alone has a band of sclerenchyma around its convex lower margin. The margins of pinnules are strongly reflexed; ecologically the leaves seem to resemble those of modern swamp xerophytes.

No adventive roots are present on the stem described here, but several isolated tetrarch Medullosan roots have been found in association. The xvlem of these Nashville roots is satisfactorily preserved (plate 3, fig. 8), but external tissues are not well displayed. Psaronius and Stigmarian rootlets generally have penetrated and distorted the softer outer tissues. The primary xylem in Nashville Medullosa roots is nearly quadrangular in cross section; the sides are only slightly extended beyond the squarish body of primary wood. The roots of Medullosa noei Steidtmann (1937) and the Medullosan roots discussed by Hoskins (1931) seem distinguishable from the Nashville roots, since the sides of the primary wood in their figures are shown to be considerably more concave in transverse sections and the protoxylem points are further extended.

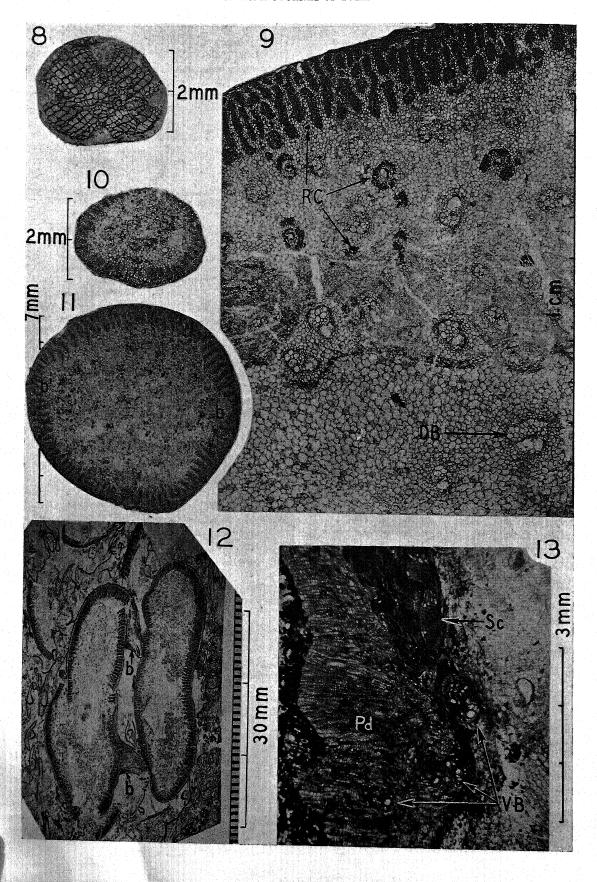
COMPARISON WITH OTHER SPECIES. 5—M. distelica is more closely related to M. anglica than to M. noei. The large size of the latter indicates an approach toward the specialization which characterized the Medullosaceae of the Permian. M. distelica is presumably intermediate in age between M. anglica and M. noei and might be expected to show intermediate characters. On the contrary, the characteristics of M. distelica are divergent in many particulars from those which might have been predicted from these previously known species, and any conception of a simple phylogenetic series may as well be discarded for the present. However a few points may be selected for comparison which seem phylogenetically significant or promising in the sense of a "Merkmals-phylogenie" (Zimmermann, 1930, p. 427).

The asymmetric development of secondary wood in Medullosa distelica may be considered first, since this feature seems to be common in the older members of the genus and most extremely exemplified by the Nashville stem. In Medullosa anglica the secondary wood on external sides of the three steles is apparently less than half the thickness of the internal secondary wood (Scott, 1899, pl. 5, phot. 1; pl. 6, phot. 5). Medullosa centrofilis De Fraine (1914) shows about the same degree of asymmetry and M. pusilla Scott (1914, pl. 13, fig. 3), although considerably smaller than the other species and hence not so subject to limiting factors of size, still shows the same asymmetric features to a significant degree. Tracings from published photographs of these species are shown in text figure 3. The type of secondary wood development has not received much attention, because it is obviously sensitive to local factors of the environment. This consideration, however, does not carry equal weight in the case of Medullosa. The fact that the four distinct older species all show a similar variation indicates that for this group the feature is phylogenetically significant. The argument possesses additional weight from the consideration that the thicker secondary wood centrally placed could not possess much survival value as a mechanical support to the plant.

Medullosa solmsii, M. stellata, and M. porosa are of Permian age and hence considerably younger

<sup>5</sup> It should be mentioned that the author has had the advantage of consulting not only Dr. Steidtmann's published description of *Medullosa noei* but also his more complete manuscript now on file awaiting publication by the Illinois Survey and dealing with this very interesting species. The extensive bibliography given there has facilitated the work in preparing the present paper, and Steidtmann's detailed description has aided in making a comparison between *Medullosa noei* and *M. distelica*.

Plate 2. Fig. 3-7.—Fig. 3. Medullosa distelica, end of one stele in transverse section showing part of primary wood more or less displaced (X1) and thickness of internal and external secondary wood (X2). From 49C (B2). (This is from the same section shown diagrammatically in text fig. 1c).—Fig. 4. Medullosa distelica, transverse section of a collateral centripetal leaf trace bundle showing tracheids (X), phloem cavity (Ph), and narrow bundle sheath. The protoxylem consists of small tracheids adjoining the phloem cavity. From 49A (T23). Magnification same as fig. 5, 6.—Fig. 5. Medullosa distelica, secretory canal containing "resin rodlet" (RR). The accompanying sclerenchyma strand (Sc) is situated adaxially from the canal. From 49A (T6).—Fig. 6. Medullosa distelica, transverse section of bundle as in fig. 4; bundle sheath is more disintegrated. From 49A (T2, 3).—Fig. 7. Medullosa distelica, the characteristic multiseriate pitting on the radial wall of a tracheid. From 49B1b2 (No. 7). Specimens in the Illinois State Geological Survey collections at Urbana.



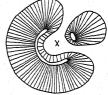
than the three English species or the American forms. The three Permian species mentioned were arboreal in structure in marked contrast to the others mentioned. This was made possible by development of a relatively great thickness of secondary wood on the external side of several steles or of one stele which came to dominate the vascular structure by curving around accessory steles and common ground tissue in the center of the stem. Thus the secondary wood again was developed asymmetrically in the principal stele or steles, but in these cases it was obviously a supporting tissue, and the principal direction of secondary growth was reversed. It is difficult to see how the woody tissue of a polystelic stem could become mechanically competent for the support of a tree by any other course of development. Since the asymmetric features of the older Medullosans were not appreciated, the full significance of arboreal development in the Permian forms also escaped particular notice. It follows, however, if stelar asymmetry is a significant feature among the older species, as it seems to be, and if these Carboniferous Medullosans are ancestral to the Permian forms, even if only in a broad sense, that the asymmetric stelar development is also of definitive significance in the latter group.

To facilitate discussion and reference to these contrasting types of secondary growth, the following terms may be useful. The types of asymmetric growth found in the older species of *Medullosa* in which the internally oriented secondary wood becomes significantly thicker, may be termed endocentric (see text fig. 2a). The contrasting type in which the externally oriented secondary wood attains the greater prominence may be termed exocentric (see text fig. 2b). It must be understood, of course, that these terms have the center of the stems (as distinct from the centers of the steles) as a reference point.

Medullosa noei and M. leuckarti seem to show about equal development of secondary wood on the internal and external sides of the principal steles, although the former is much more regular in its stelar development than the latter. If the exocentric or endocentric growth of secondary wood is accepted as a character of importance within the genus, then we must think of M. noei and M. leu-

ckarti as intermediate in the series. The geologic ages of these two forms also seems to lend support to this interpretation. Thus, on the basis of the type of secondary growth we seem to have an illustration of evolution from plants with inefficient stelar supporting tissues to plants of considerable efficience in this one particular within the basic





a. Endocentric(x-center of stem)

b. Exocentric (x-center of stem)

Text fig. 2. a. Diagrammatic representation of the steles of a tristelar stem in which the internal production of secondary wood predominates. b. Diagrammatic representation of two stelar forms which illustrate predominance of external secondary wood. Radiating lines represent secondary wood of each stele; open area within the secondary wood represents primary wood or mixed "pith."

limitations imposed by a less flexible polystelic structural plan.

Medullosa distelica provides no significant evidence concerning the origin of polystely in this group. The presence of only two instead of three or more steles is certainly a valid species character, but it is not clear whether this is a step toward simplification of the polystelar condition or an ancient hold-over from a primitive less complex stelar constitution. In the past several authors have suggested a possible alliance between the Cladoxyleae with many "steles" (of endocentric configuration) and the Medullosaceae. However, Bertrand (1935) has shown conclusively that Cladoxylon is the stem which bears certain Zygopterid phyllophores (Clepsydropsis) so that relation to the Cycadofilicales is no longer plausible. De Fraine (1912) has suggested that the monostelic Sutcliffia more nearly approximates the primitive stelar form of the Medullosaceae. This still seems

Plate 3. Fig. 8-13.—Fig. 8. Medullosa rootlet, transverse section. The nearly square outline of primary xylem is clearly shown. From 113-3 (T4).—Fig. 9. Myeloxylon petiole associated with Medullosa distelica. The sclerotic strands of the rind are more radially elongated in this sector than in the holotype of M. distelica. Secretory canals (RC) are numerous both with and without sclerenchyma strands in conjunction. Black contents of canals are "resin rodlets." Paired leaf trace bundles occur along the horizontal mid-line of the photographs, and a double bundle (DT) in course of division is shown lower at the right. From 44A3.—Fig. 10. Tertiary petiole of Myeloxylon. The hypodermal sclerenchyma has assumed a dorsiventral arrangement. Magnification is the same as in figure 9. From 44c.—Fig. 11. Secondary Myeloxylon petiole. The parenchymatous bays in the marginal sclerenchyma at b and b' represent points at which tertiary petioles arise. From 64B2b (B1).—Fig. 12. Myeloxylon petioles which probably represent main rachides further removed from their stem than the sector shown in fig. 9. The small black specks within the marginal sclerenchyma are nearly all resin rodlets. The petiole at the left is giving off two secondary petioles (b). Many transverse and oblique sections of Lepidophyllum sp. are also present. From 62D1 (T1).—Fig. 13. Medullosa distelica, sector from the specimen illustrated in figure 2. Leaf traces (VB) have been enveloped by the periderm (Pd) and somewhat disarranged. Internal sclerenchyma strands (Sc) of an adjacent leaf base are shown close outside the periderm in the upper part of the figure. From 49A (T4). Specimens in the Illinois State Geological Survey collections at Urbana.

to be an acceptable interpretation. Nevertheless, in consonance with the next point to be discussed, it seems more tenable to consider the distelar condition in the new Nashville Medullosa as reduced from a more polystelar type rather than as directly derived from an ancestry reminiscent of Sutcliffia. It is also apparent that the extreme endocentric asymmetry found in M. distelica is not a primitive feature. For these reasons, in spite of the apparent simplicity in stelar construction, M. distelica should be considered as a more advanced form than M. anglica.

The amount of secondary wood accompanying leaf traces as they leave the stele may be sig-This is suggested by the organization found in Sutcliffia which on other grounds may be considered as primitive within the family. The leaf traces in mature stems of Sutcliffia are qualitatively similar to the central cylinder and are poorly differentiated from it even to the point of rejoining. For convenience they have been termed "meristeles," but the small leaf trace bundles are produced from them according to De Fraine (1912), and because of this they are interpreted as foliar rather than as essentially cauline structures. They possess a considerable development of secondary wood the same as the central cylinder along this "meristelar" portion. Medullosa anglica develops a significant amount of secondary wood radially around the main leaf traces for several centimeters of their proximal extent before they break into smaller collateral bundles. None of the other species of Medullosa are known to possess secondary tracheids along leaf traces, and except for this feature the leaf supply of M. distelica seems to have been quite similar to that of M. anglica. Incomplete as this information is, it may indicate that the differentiation between the megaphyllous leaf and stem was still in progress during evolution of the older members of this family.

The position of the protoxylem may prove to be useful in comparing members of the Medullosaceae, although definite information is not available for several of the species. Sutcliffia is clearly exarch. Medullosa centrofilis is exarch or slightly mesarch, M. anglica is mesarch, and M. distelica is probably mesarch, too. M. noei was not determinable for this characteristic in Steidtmann's material, but from better preserved specimens which the author has examined it is seen to possess protoxylem centrally located in some of the tracheid nests of the primary wood, a definitely mesarch condition. There thus seems to be a tendency for the protoxylem to become progressively less associated with initiation of secondary growth.

### THE ANGLICA GROUP OF MEDULLOSA

Weber and Sterzel's "Beitrage" (1896) approaches closest to a monographic treatment of members of the Medullosaceae. Their studies have dominated the taxonomy of the group in recent years,

although the species they dealt with are chiefly of Permian age. Their contribution is confined in the main to four species; M. porosa (which probably is to be considered the genotype, since it seems to have been the first described), M. stellata, M. solmsi, and M. leuckarti. Weber and Sterzel named different growth forms of the first three of these, based chiefly on the amount of secondary wood. The different forms were grouped under their respective specific designations, each as a "Formenkreis" of one species by Weber and Sterzel. M. leuckarti had no distinct growth forms, although it also was termed a "Formenkreis." Scott gave the most thorough morphologic treatment of any of the species when he described Medullosa anglica (1899) from the Lower Coal Measures of Great Britain; several years later he described Medullosa pusilla (1914) from about the same horizon. When De Fraine (1914) described M. centrofilis (also of Lower Coal Measures age), she suggested that these three older species be grouped as the Medullosa anglica "Formenkreis" or "form-cycle." As a temporary expedient this informal grouping served to point out closer relationships within the genus.

Since the forms described in this paper show many features in common with those included in the M. anglica "form-cycle," it does not seem necessarv now to classify the Anglica group on such a provisional basis. This alliance now includes the three English species and two or more American types which are rather definitely distinguished among themselves, but all present a sharp contrast to the exocentric Permian forms. Weber and Sterzel's "Formenkreis" is apparently a convenient designation for a single somewhat variable fossil plant species, but it is inappropriate for the more extensive assemblage discussed here. It is proposed, therefore, that this older alliance of Medullosa species be set apart with the rank of a subgenus and, since it seems to constitute a natural group, that it be accorded formal taxonomic status. This subgenus may be diagnosed as follows:

Subgenus Anglorota subgen. nov.—Plants small to medium size, some possibly of creeping or climbing habit. Stems relatively weak, supporting tissue notably present in the "hypoderm" of leaf bases and petioles. Polystelic; the few (2-4) steles which dominate the vascular system are approximately equivalent; accessory vascular structures (star rings, etc.) few and relatively inconspicuous or altogether absent. Secondary xylem present but insufficient to function as the chief supporting tissue; characteristically grows thicker on the internal sides of the individual steles (endocentric). Internal periderm present and generally bandlike. Stem completely sheathed by relatively large decurrent petiole bases arranged in a simple spiral phyllotaxy. The name Anglorota is essentially a latinization of anglica cycle-i.e., form-cycle.

Type species: Medullosa anglica Scott (1899). Thiessen's Medullosa is closely allied with M. anglica but it deserves at least varietal distinction.

It is therefore proposed to designate it in honor of the late Reinhardt Thiessen. This specimen has been made available through the courtesy of George Sprunk of the U. S. Bureau of Mines, and the author gratefully acknowledges his kind cooperation. The description may be given as follows:

Medullosa anglica var. thiesseni var. nov.—The specimen consists of a slender columnar segment of stem about 14 cm. long and 20 mm. in diameter. The stelar portions and the innermost cortical tissues are preserved by pyrite and hematite with relatively little crushing or distortion. It is longitudinally ribbed and somewhat triangular. Externally there are no indications of leaf bases leaving the stem, although it may be that these were differentiated further out in the more marginal portion which is not preserved. A continuous narrow periderm band separates the stem into cortical and stelar portions.

The preserved cortical part varies from about 4 to 7 mm. thick outside the periderm. The numerous collateral bundles of the cortex seem to lack any particular orientation. From 34 bundles in one sector (including about half of the stem) 6 are obviously double preparatory to dividing, and 25 are simple; the others are intermediate in con-Nine have the protoxylem directed formation. toward the center of the stem, 10 have the protoxylem directed toward the periphery, and the rest are oriented tangentially with the protoxylem on one side or the other. A narrow sclerenchymatous bundle sheath extends around the xylar side of each bundle. Secretory canals with or without a small crescentic accompaniment of sclerenchyma are numerous, 66 having been counted in the sector mentioned above. Sixteen of these have associated sclerenchyma strands located on the internal side. In none of the sections studied has any definable sclerenchymatous zone in the cortex been observed which might be interpreted as the internal part of a leaf base.

The internal periderm is very narrow, twisted, and continuous, similar to that in *M. centrofilis*. It probably does not exceed 10 cells in thickness at any point. Peculiarly, at several points resin rodlets are completely enveloped by the periderm.

Pericycle and phloem within the periderm band are poorly preserved. In a few areas parenchymatous cells can be observed, so it seems likely that if any fibrous elements had been present in this region that they, too, would be seen. Resin rodlets are very numerous especially in the zone between the periderm and the steles, and a row of them is also present between the woody cylinders. These rodlets are about the same size as those in secretory canals outside the periderm, though a few are smaller.

Three steles are present, which apparently run the length of the specimen without significant change. They are smaller, more compactly spaced,

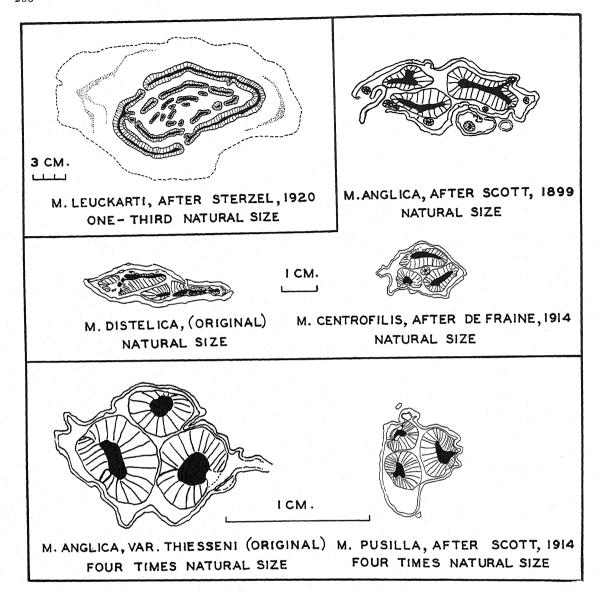
and are much more regular in outline than those in Scott's specimens of M. anglica (text fig. 3). The three cylinders are not the same size, although they are obviously morphological equivalents. They are about 6.6  $\times$  5.2 mm., 6  $\times$  3 mm., and 5  $\times$  3 mm. in diameter, the first measurement in each case being tangential and the last radial to the stem as a whole. This size variation seems consistent throughout the specimen, and it is interesting to note that a similar variation is suggested by cross sections of each of the English forms as well (text fig. 3). The steles of our new variety are asymmetrical (endocentric) as in M. anglica but not quite so extreme. The primary wood follows the same contours as the secondary wood and occupies a little less than a third of each dimension. The primary tracheids seem relatively more numerous than in M. distelica, but precise details are hard to make out, and one cannot be sure of the position of the stelar protoxylem.

The details reported have been observed on relief polished sections of this specimen, since the preserving minerals are opaque and thin sections are of course out of the question. Possibly if more sections were cut, additional details might be added. Thiessen's original illustrations (1920a, 1920b) may be referred to for further elucidation of this variety.

The facts presented seem sufficient to distinguish Thiessen's specimen from M. anglica. If comparable material is discovered in the future at a known horizon, it may even be considered specifically distinct. As matters stand, a varietal name seems sufficient as an adequate means of reference. Medullosa anglica var. thiesseni is significant, since it provides indisputable evidence linking the Anglorota species of America with those known only from Europe.

The other species included in this subgenus are M. pusilla Scott (1914), M. centrofilis De Fraine (1914), M. distelica Schopf described on a previous page, and possibly M. noei Steidtmann (1937). Scott (1923, p. 186) mentions sections in the Renault collection at Paris bearing the name "Heterangium geriense" which he says is a Medullosa belonging to the Anglica group. He suggests that "this species may bear the name Medullosa geriensis," but so far as the author is aware, it is still undescribed. Until some one describes it, "M. geriensis" must be treated as a nomen nudum. However, the sections referred to are reported to come from "the upper coal measures of the Ride de Gier, near St. Etienne," and consequently this form may be geologically the youngest of the Anglorotae.

Features of stelar form and arrangement of Anglorota species are shown by reasonably accurate line tracings in text figure 3. Medullosa noei is larger than any of these and differs in several other particulars. It may be desirable to place M. noei and M. leuckarti together somewhat separate from the rest of the genus, but this must await further study. No tracing of M. noei is given, because no very ex-



Text fig. 3. Stelar systems of Anglorota Medullosans and of Medullosa leuckarti. Primary wood area blacked in; secondary wood lined, lines follow the rows of secondary tracheids. Outer band in each (except M. leuckarti) represents internal periderm.

cellent entire stems are available. However, M. leuckarti is shown for comparison, although it is unquestionably more advanced than M. noei or any of the older forms.

### SUMMARY

Medullosa distelica sp. nov. and petioles, leaflets, and roots associated with it have been described. The stem possesses two highly asymmetric (endocentric) steles. The associated petioles possess a somewhat different type of sclerotic rind than the stem, although they are probably derived from the same species. The roots seem distinguishable from those known from other species of Medullosa and

probably were similarly adventive, although none were found in connection. A few points—viz., the asymmetrical growth of the steles, the leaf trace accompaniment of secondary wood, and the location of the protoxylem in the stem—have been compared with other species. It is pointed out that the type of secondary wood formation (endocentric or exocentric) may be significant within this group for taxonomic purposes. Although M. distelica is simpler in its gross stelar anatomy than M. anglica, this condition is regarded as derived rather than primitive.

The Medullosa "form-cycles" have been discussed briefly. The older species, which include the M. anglica "form-cycle" and American forms, are

considered sufficiently distinct from the rest of the genus to be recognized as a subgeneric group designated as Anglorota. A specimen illustrated earlier by Reinhardt Thiessen shows sufficient agreement with M. anglica to be described as a variety of that

species and indicates best how closely American and European forms are related.

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### POLLINATION AND ITS INFLUENCES ON THE BEHAVIOR OF THE PISTILLATE FLOWER IN VALLISNERIA SPIRALIS <sup>1</sup>

### S. B. Kausik

Vallisneria spiralis L., a submerged aquatic belonging to the order Helobiales, has long been known as a classic example of a unique type of pollination first described by Kerner (1891). Twenty years have elapsed since Wylie (1917) studied the American form<sup>2</sup> of this plant and gave a vivid account of the processes involved in the transfer of pollen from the staminate flowers to the stigmatic lobes of the pistillate flowers on the surface of the water. The following account presents the same aspect in the life history based on a form of Vallisneria which grows in India and shows certain essenteria

<sup>1</sup> Received for publication January 10, 1939.

The writer wishes to state that he enjoyed correspondence with Professor Robert B. Wylie, of the State University of Iowa, U. S. A., who has offered helpful suggestions; he wishes also to thank Professor M. A. Sampatheumaran, of the University of Mysore, Bangalore, India, for his kindness throughout the progress of this study and for helpful criticism at various times.

<sup>2</sup> In discussing the diagnostic character of the American form of Vallisneria, Fernald (1918) states that treated as a species it is V. americana Michaux, or as a variety it would be V. spiralis, var. americana (Michx.) Torr.

tial departures from the earlier accounts by Kerner and Wylie. This paper also includes a study of the behavior of the pistillate flowers both prior to and after pollination.

The writer recently noticed large patches of Vallisneria spiralis growing in a small lake adjoining the Government Horticultural Gardens, Lal Bagh, Bangalore, India. Many pistillate flowers had reached the surface of the water and were disposed more or less slantingly, exposing their stigmatic lobes and causing slight depressions of the surface of the water. During quieter periods the staminate flowers glided into these depressions and were grouped together in contact with the stigmatic lobes (fig. 1). But with the formation of waves, the clusters of staminate flowers generally drifted away from the pistillate flowers. This observation suggested that there might be departures from the account of pollination by Wylie (1917). Further, the flowers themselves were found to be rather different from his descriptions. Consequently, a detailed study of pollination and also of the structure of the flowers was undertaken with plants in natural surroundings as well as in the laboratory.

The staminate flower is about 0.6 mm. across the recurved sepals, which are three in number and of which one is smaller. There are two stamens with slender filaments spreading apart at right angles. Each stamen bears distally, when the anther has dehisced, a mass of large pollen grains. The two masses of pollen grains of a given flower project beyond the margins of the sepals (fig. 2-4) so that the

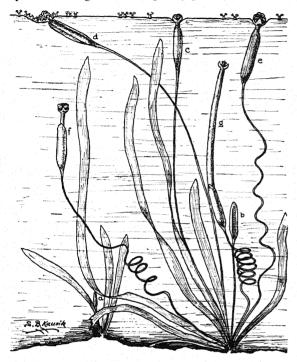


Fig. 1. General drawing of  $Vallisneria\ spiralis\ L.$ , showing a number of freely floating staminate flowers on the surface of water and pistillate flowers in various stages of development, marked a to g.

pollen grains may immediately come in contact with the stigmas of the pistillate flower. In addition to the two stamens, the staminate flower has a rudimentary petal and a small staminodium placed on opposite sides. The staminate flower agrees in many respects with that figured by Kerner (1891), but differs in one respect-namely, that the sepals are unequal, while Kerner's figure shows them as equal and symmetrically disposed. On the other hand, a comparison with the American form studied by Wylie (1917) shows more striking differences in the floral parts. The sepals are of course similar in both. but the pointed hairs described by Wylie as occurring at the base of the stamens are absent. The most important difference, however, is that the stamens, instead of being close together and upraised in the center of the flower, forming a single mass of pollen grains, are divergent with distinct pollen masses.

There are also differences in the structure and development of the sporangia themselves. Rangasamy

(1934) points out that each anther in Vallisneria (available locally) has usually four sporangia which are derived from independent archesporial masses, but suggests that there might be slight variations. On the other hand, Witmer (1937), in a recent study of the American form, states that each anther has normally only two sporangia and that these are formed from a common archesporium later becoming separate as growth in the stamens proceeds.

The pistillate flower also shows important differences, in size as well as in structure, when compared with that in the American plant. It has a slender inferior ovary measuring about 12 to 14 mm. in length at the time of pollination (fig. 6), while Wylie states that it is about 20 to 25 mm. in the American form. The spathe invests the ovary almost up to the base of the spreading sepals, as stated by Wylie, and later forms in the developing fruit only a partial basal investment. On the other hand, the figure by Kerner (1891) shows that the spathe leaves a considerable portion of the ovary below the sepals quite naked even at the time of pollination. Three rudimentary petals are found inside the sepals and attached at the base of the style. No staminodia are seen, but these are said to be present in the American form by both Wylie (1917) and Witmer (1937). There are three stigmas, each of which is bifid with two leaf-like fleshy expansions curling slightly outside and between the sepals to expose the inner surfaces, which are densely clothed with stigmatic hairs (fig. 7). Wylie reports that the bifid stigmas are sharply coiled in the American form when the flowers are fully open and that these coils protrude between and outside the sepals. This disposition of the stigmas in the American form favors contact between the stigmatic lobes and the pollen masses. In this connection it is of interest to note that Professor Wylie, in a recent personal communication, writes: "I am inclined to suggest that while the American Vallisneria does not have the stamens separated, this possible handicap is more than compensated for by the extensive stigmas which are coiled and protrude between and outside the sepals. assuming the recognition you have given to the depression of the surface film. They may possibly be as fully exposed to pollen contact as in your form in which as you say the stigmas are curled outside the sepals."

It is thus seen that the two forms are structurally very different in their flowers. While the pistillate flower in the Indian form is only half as long as that in the American form, the staminate flowers do not appreciably differ in size, being less than a millimeter across in either. The size relationship then, between the staminate and the pistillate flowers in the Indian form, is very different from that in the American form; this disparity is perhaps correlated with the difference in pollination noted for these two forms.

The plants grow along the margins of lakes in shallow water where the depth may not exceed usually about one and a half or two feet. The leaves are strap-shaped and attain a length of approximately a foot or a little more. The American plant, on the contrary, appears to be much larger, with leaves up to a meter or more in length. The pistillate flower develops under water and is brought to the surface at the time of pollination by the elongated slender scape (Fig. 1d). The latter forms some surplus length which places the pistillate flower in a slanting position on the surface film of water. The floral parts of the flower are exposed on the surface and on account of the weight of these, the surface film forms a slight cup-like depression about each pistillate flower. At this time staminate flowers, floating on the surface, are moved along by currents or wind and may reach these depressions, when they tumble down and perforce strike against the stigmatic lobes of the pistillate flowers (fig. 1d). In this act a quantity of pollen is immediately shed on the stigmatic lobes, which upon examination show loose pollen grains, as large as 50  $\mu$ , sticking to their receptive surfaces. When, however, the water is disturbed, as frequently happens by the formation of strong waves, the pistillate flowers may suffer a temporary submergence, at which time the associated staminate flowers generally lose their contact with the stigmas and drift away. But, with the passing of the wave, the pistillate flowers are again exposed in the surface depressions. The transfer of pollen to the stigmatic lobes proceeds favorably during the short intervals when the surface of water is fairly quiet between successive waves. While many staminate flowers have merely a chance of "touch and go," the majority of them perhaps never reach the pistillate flowers but are washed ashore.

While the present observations are in general agreement with the details of pollination suggested by Wylie (1917) and strongly support the emphasis laid by him on the surface film in aiding pollination, the submergence of the pistillate flowers by the formation of waves does not appear to the writer to be a prime factor in the Indian form. In describing the events in pollination, Wylie suggests that submergence is undoubtedly beneficial to pollen transfer in the American form. However he notes elsewhere in the paper: "Attention should be directed to the fact that any degree of depression is helpful, and that complete submergence, although this probably occurs frequently, is not necessary to adequate pollination." The writer wishes to emphasize that pollination in the Indian form can go on uninterruptedly in the absence of submergence and in fact takes place largely during periods when the surface is not agitated by waves and strong ripples. It is, however, possible that submergence is indirectly helpful to some extent, in that it causes a dislodgement of old staminate flowers from the stigmatic lobes and thus offers chances for fresh staminate flowers to function in pollination.

The different degrees of importance attached to the submergence of the pistillate flowers in the American and the Indian forms may perhaps be properly ascribed to the relative size and structural differences in the flowers of these two forms. The divergent anthers appear to be perfectly suited to bring their pollen into immediate and direct contact with the stigmas in the surface depressions even

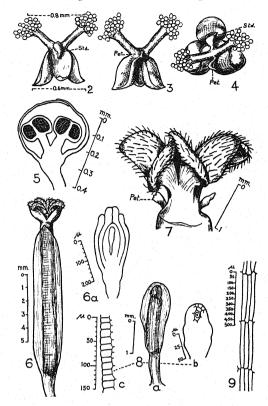


Fig. 2-9. Camera lucida tracings of the staminate and pistillate flowers at different stages of development.— Fig. 2-4. Staminate flower in different views to show details of floral parts.—Fig. 5. Outline drawing of longitudinal section of an unopened staminate flower to show divergent stamens.—Fig. 6. Pistillate flower at the time of pollination.—Fig. 6a. An ovule from the same, showing embryo sac in outline.—Fig. 7. Stigmatic lobes of the pistillate flowers.—Fig. 8. a, young pistillate flower; b, its ovule at the megaspore mother cell stage; c, the epidermal cells of the scape of same.—Fig. 9. Epidermal cells of the scape of a pistillate flower at the time of pollination to show the elongated cells. Pet., Rudimentary petal; Std., staminodium.

when the water is fairly still. Further, the pistillate flower is much smaller than in the American form, and it therefore presents a much smaller area at the surface of water for contact with the staminate flowers; furthermore, during submergence it is not capable of retaining the staminate flowers in such large numbers or as generally as in the American form, which has a much larger area of depression and the stigmas are sharply coiled.

The earlier work of Kerner (1891) appears to be, as already suggested by Wylie (1917), "highly generalized, and perhaps intended to convey only a general account of the pollination in this plant." Kerner

has ignored the special agency of the surface film in the process of pollination and figures a level water surface with the pistillate flower held high straight above water as if by a stalk. The cup-like depression, which is so essential in capturing and retaining temporarily the staminate flowers, was entirely overlooked by him. His account of pollination appears to be based on an unsound principle, for the surface depression is a natural phenomenon formed on account of the purely physical property that the surface film of water has in relation with any floating object that is not wetted by water. Further, the arrangement of the flowers themselves on a level water surface, as depicted by him, does not seem to be conducive to adequate pollination.

INFLUENCES OF POLLINATION ON THE BEHAVIOR OF THE PISTILLATE FLOWERS.—Turning now to the second aspect of this paper-namely, the behavior of the pistillate flowers, prior to and after pollination—the following is a description of some features noticed by the writer. The pistillate flower develops under water, and at the stage when its ovules are in the megaspore mother cell condition, it is a little over 2 mm. long, with a very short stalk measuring about a millimeter (fig. 8a). The stalk gradually increases in length until it brings the pistillate flower to the surface of the water. The increase in length is largely due to an elongation of the individual cells of the scape; in a number of instances it was found by measurements that the cells had increased by about 40 to 50 times their original length (fig. 8c, 9). It may be noted here that the pistillate flower of Elodea Wylie (1904) also reaches the surface of the water by cell elongation, at the time of pollination, of that part of the flower above the ovary.

When the pistillate flower has just reached the surface of the water, it is nearly vertical but later gradually assumes a more or less horizontal position by the further increase in the length of the scape. The stigmatic lobes spread out in about two to four hours, and pollination is effected in favorable circumstances in about four to six hours. After pollination the scape of the pistillate flower undergoes a spiral torsion, which is first evident at the base of the ovary. This is followed by the formation of a number of coils in the scape. These coils then draw closer and tighten, and the pistillate flower retreats under water, where the fruit is developed. Müller (1878) pointed out that the anatomical structure of the scape includes two vascular strands, of which one is smaller than the other; if the growth of these two strands, as suggested by Svedelius (1904), is different, the result would be a coiling of the scape. In a quiet aquarium where the plants were kept for observation, it was found that subsequent to pollination the pistillate flower had retreated under the surface of water by a centimeter in about three hours, and in the course of another three hours it was 7 cm. below. This rate of retreat of the pistillate flower need not, however, hold good in natural surroundings.

A change in the depth of water, as, for example, when the plants are transferred from their natural surroundings to aquaria, brings about a corresponding change in the length attained by the scapes of the developing pistillate flowers. In the case of a plant which had a number of pistillate flowers some of which had already developed into fruits in the natural surroundings, with the scapes attaining their normal length of about 46 cm., one developing young flower was marked for observations. The plant was then transferred to the aquarium where the depth of water was 16 cm.; when the marked pistillate flower finally came to the surface of the water in the aquarium, it had developed a scape only 18 cm. long. Chances for pollination were immediately offered by introducing a number of staminate flowers on the surface of the water after the stigmas had completely opened; in about three hours, during which time pollination occurred, the flower commenced to retreat under water.

If, on the other hand, pollination is prevented, as was done in other similarly marked pistillate flowers, they remain on the surface of the water for a prolonged period after which they begin to retreat, rather slowly, by the formation of only a few loose coils in the scape. Sometimes, however, the length of the scape may continue to increase afresh even after the pistillate flower is completely exposed on the surface of the water and has been lying thus for some time; in one instance it was found to have developed a scape fully 40 cm? long while the water in the aquarium was only 16 cm. deep. When chances for pollination were provided, even at this late stage, the pistillate flower behaved normally and promptly developed into a fruit. In the complete absence of pollination the flowers sank only a little under water, and the formation of the fruit was altogether suppressed. Wylie (1917) states that "the scapes will coil somewhat without pollination, so the retreat of the seed-bearing flower is the final step in pollination if the pollen-bearing flowers are present." Under normal conditions following pollination the pistillate flower sooner or later retreats under water and forms the fruit.

Discussion.—In considering the importance of the surface film in pollination in Vallisneria it is desirable to point out the few other recorded instances of a similar nature. Cowles (1911) mentions the film pockets formed on the surface of the water but does not enter into a further discussion. Wylie (1904), in discussing the phenomenon of pollination in Elodea canadensis, has fully described the surface depression of the water about each flower into which slide the floating pollen grains. The case of Elodea is somewhat different from that of Vallisneria, for in the latter it is the free floating staminate flowers themselves and not pollen grains scattered loose on the surface of the water which are gathered in the surface depressions. The process of pollination, however, is essentially similar in both in that the surface depressions play an important rôle. Svedelius (1904), on the other hand, does not make

any reference to the possible part played by the surface film in the pollination of Enalus acoroides, in which he states that the cross folds of the long and delicate petals of the pistillate flowers serve to capture and retain the staminate flowers as they arrive floating on the surface of the water. But Enalus presents a modified case in that the plants live in the open sea where the currents are very strong and may often reach a speed of 5 to 6 knots in an hour (Svedelius, 1904), so that a very effective device for retaining the staminate flowers for pollination would be of advantage. The most complete and also the latest treatment of the subject of surface pollination is by Wylie (1917), who has emphasized the importance of the surface film in the pollination of Vallisneria. Kerner (1891), as was pointed out by Wylie (1917), failed to note any surface film relations in the pollination of this plant.

The present account of pollination in Vallisneria. the Indian form of which differs very considerably in several essential features from the American form, is in general corroborative of the observations of Wylie (1917). It differs only in one respect which appears to the writer to be of sufficient importance to be stressed—namely, in the influence of submergence of the pistillate flowers in aiding pollination. As already noted, it seems to be the size and the structural differences in the flowers in the Indian form that are responsible for this difference. A reinvestigation of the form studied by Kerner will doubtless show it behaved very much like the Indian form, for our species seems to agree more closely with the Vallisneria of Europe in the plan of the staminate flowers which have the widely divergent stamens figured by Kerner and noted by Svedelius (1932). The pistillate flowers are also more like the European type and are much smaller than in the American form. The differences between the various forms are so marked as to point out the desirability of a taxonomic review of the genus. This, however, does not fall within the scope of the present study.

In concluding the present study of pollination in *Vallisneria spiralis*, the remarks of Wylie (1917) in the introduction to his paper on this interesting

phase in the life history of the plant may be restated: "Living vegetatively as a submersed aquatic, its dioecious flowers are brought together at the surface of the water in most ingenious fashion. These highly specialized flowers present the strongest contrasts, not only in size and structure, but in behavior as well, and give this plant its rank as one of the climax types with respect to floral differentiation. Specializations of such evident advantage for crosspollination in a form so admirably situated for vegetative propagation seem to emphasize the importance of sexuality, or at least of seed production, in the higher plants."

#### SUMMARY

The account of pollination in Vallisneria spiralis presented here differs slightly from that given by Wylie (1917). While submergence of the pistillate flowers may further pollination in the American form, it is not of such significance in the smaller Indian form but may be indirectly helpful in bringing fresh staminate flowers into the depressions about the pistillate flowers. The present account, otherwise, supports the observations of Wylie with regard to the part played by the surface film forming the depressions to capture the staminate flowers. Since the Indian form is more like the European, the present findings indicate that the account of pollination by Kerner (1891) may be regarded as somewhat generalized. The differences in flower structure between the various forms from different regions call for a taxonomic review of the genus.

The second part of the paper concerns the influences of pollination on the behavior of the pistillate flowers. The period ordinarily open to pollination is several hours, and the subsequent rate of retreat of the pistillate flower under water is very slow at first, less than a centimeter per hour. Pistillate flowers in the lake had scapes 46 cm. long; when the plants were transplanted to an aquarium with water 16 cm. in depth, the scapes attained a length of only 18 cm.

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# RELATION OF LATITUDE TO CERTAIN PHASES OF THE GROWTH OF TIMOTHY $^{\rm 1}$

## Morgan W. Evans

AN EXPERIMENT was conducted for the purpose of determining the relation of latitude to certain phases of the growth of early, medium, and late timothy (Phleum pratense L.) and to the progress from one latitude to another of their seasons for heading, blooming, and maturing (Evans, Allard, and McConkey, 1935). This experiment was conducted at ten stations at different latitudes, as shown in table 1. In addition to the recorded variations in latitude, data are presented in regard to the longitude, altitude, mean annual temperature, and the length of the longest day at each station; no information was obtained in regard to variations in soil type, rainfall, humidity, or other factors than those mentioned which may have affected the results. In this report the results of a second, related experiment are also presented, which show the effects of latitude upon the yields of hay produced by early, medium, and late strains of timothy.

Because of the nature of this investigation, the results presented in this report have been made possible only through the cooperation of a number of widely separated collaborators. Data were obtained by Professor George E. Ritchey, associate agronomist, Division of Forage Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, Gainesville, Florida; Mr. R. E. Stitt, assistant agronomist, Division of Forage Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, Statesville, North Caro-

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The author is indebted to Professor George T. Jones, Department of Botany, Oberlin College, Oberlin, Ohio, for helpful suggestions and criticisms. lina; Mr. H. A. Allard, senior physiologist, Division of Tobacco and Plant Nutrition, Bureau of Plant Industry, United States Department of Agriculture, Washington, D. C.; Mr. J. E. Ely, agent, Division of Forage Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, North Ridgeville, Ohio; Professor O. Mc-Conkey, Division of Farm Crops, College of Agriculture, Guelph, Ontario, Canada; Mr. D. H. Holt, assistant in agronomy, Branch Experiment Station, Ashland, Wisconsin; Dr. G. F. H. Buckley, agrostologist, Dominion Experimental Farm, Brandon, Manitoba, Canada; Mr. T. M. Stevenson, agrostologist specialist, Dominion Forage Crops Laboratory, Saskatoon, Saskatchewan, Canada; Mr. W. D. Albright, superintendent, and Mr. E. C. Stacey, assistant superintendent, Dominion Experimental farms, Sub-station, Beaverlodge, Alberta, Canada; Mr. Albert Lawrence, officer in charge, Dominion Experimental Farms, Sub-station, Fort Vermilion, Alberta, Canada; Dr. A. Daane, agronomist (deceased), Everglades Experiment Station, Belle Glade, Florida; Mr. C. G. Marshall, Acclimatization Garden, Division of Foreign Plant Introduction, Bureau of Plant Industry, United States Department of Agriculture, La Jolla, California; Professor E. N. Fergus, Department of Agronomy, Agricultural Experiment Station, Lexington, Kentucky.

CONDITIONS DEPENDENT UPON LATITUDE WHICH AFFECT THE GROWING HABITS OF TIMOTHY.—According to the older biological writings, temperature is the most important factor in determining the distribution of plants and animals (Shelford, 1932,

Table 1. Latitude, longitude, altitude, mean annual temperature, and length of the longest day at each station where tests were conducted."

Station	Latitude	Longitude	Altitude	Mean annual tem- perature	Length of longest day
	Degrees	Degrees	Feet	Degrees F.	Hours
Gainesville, Fla., U. S	29.6	82.3	165	69.8	14.1
Statesville, N. C., U. S	35.8	81.9	950	58.8	14.6
Washington, D. C., U. S. (Arling-					
ton Experiment Farm)	38.9	77.1	50	55.6	14.9
North Ridgeville, Ohio, U. S	41.4	82.0	750 <sup>b</sup>	49.3	15.1
Guelph, Ont. Can	43.5	80.3	1150	43.4	15.4
Ashland, Wis., U. S	46.6	90.9	648	40.6	15.9
Brandon, Man., Can	49.8	99.9	1200	33.5	16.3
Saskatoon, Sask., Can	52.1	106.6	1690	33.3	16.7
Beaverlodge, Alta., Can	55.2	119.3	2484	35.1	17.4
Ft. Vermilion, Alta., Can	58.4	116.0	950	27.3	18.3

<sup>\*</sup>The data for stations in the United States were obtained from the Weather Bureau of the United States Department of Agriculture, Washington, D. C. The data for the stations in Canada are from the Meterological Service of Canada, Department of Transport, Toronto 5, Ontario, Canada.

b Approximate.

p. 147). According to Kendeigh (1932), Reaumur (1735) calculated the season for the appearance of plants and animals by summing the accumulated degrees of temperature above freezing. Later Merriam (1894, 1899) mapped life zones in North America. In determining the boundaries of these zones, Merriam used as effective degrees of temperature only those above 43 degrees F. (6 degrees C.), which DeCondolle in 1835 had shown to be the threshold temperature at which wheat will germinate (Kendeigh, 1932). In Hopkins' Bioclimatic Law (Hopkins, 1918), as in earlier theories, a predominating influence was assigned to temperature. According to this law, if other conditions, such as altitude, topography, etc., are uniform, the time of blooming of any species of plant should progress from south to north, along the same degree of longitude, at the uniform rate of one-fourth of a degree of latitude each day.

Failure of the older theories to predict accurately the progress of the season.—Shelford (1932) and Kendeigh (1932) have shown that the life zones described by Merriam do not coincide with the plantanimal communities recognized in modern ecology.

Table 2 contains records for 1928 (Evans, 1931) of the dates upon which florets began to bloom on plants of an early selection of timothy at six stations from Savannah, Georgia, to Fairbanks, Alaska. The table also shows the predicted dates when the plants at the stations north of Savannah should have begun to bloom, according to Hopkins' Bioclimatic Law. In general, the farther north the plants were grown, the greater numbers of days did they bloom before the predicted dates. These results indicate that some factor other than temperature, and not recognized in the Bioclimatic Law, had an important influence upon the time of blooming.

Relation of temperature to the time of blooming.—The effects of seasonal variations in temperature upon the time of blooming of ordinary American timothy is shown in data obtained at the Timothy Breeding Station<sup>2</sup> at North Ridgeville, Ohio,

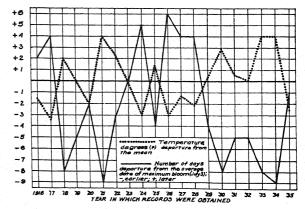


Fig. 1. Relation of the mean temperature during April, May, and June to the date the maximum percentage of plants were in full bloom. The records were obtained from 1916 to 1935.

in each year from 1916 to 1935, excepting 1919. Figure 1 shows the average number of degrees F. daily departure from the mean temperature during April, May, and June;<sup>3</sup> it also shows the number of

<sup>2</sup> Conducted cooperatively by the Division of Forage Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture and the Department of Agronomy, Ohio Agricultural Experiment Station.

<sup>3</sup> The temperature data were obtained by the United States Department of Agriculture Weather Bureau Station at Cleveland, Ohio, approximately 20 miles east of and at a not greatly different elevation than the Timothy Breeding Station.

Table 2. Dates in 1928 when the first florets bloomed on plants of timothy number 11902 at stations at different latitudes, and the dates when they should have bloomed according to Hopkins' Bioclimatic Law.

Station	Lati- tude	Longi- tude		Length of long-est day	Date first florets bloomed	Predicted date of bloom according to Hopkins' Bio- climatic Law	
	Degrees	Degrees	Feet	Hours			
Savannah, Ga	32.1	81.1	42	14.3	May 31		
Jackson, Tenn		88.8	450	14.6	June 11	June 12	1
North Ridgeville, O		82.0	$750^{\rm b}$	15.1	June 26	July 14	18
Fargo, N. D		96.8	903	15.8	July 2	July 25	23
Edmonton, Alta		113.6	2183	17.4	July 7	August 20	44
Fairbanks, Alas	2.0	147.6	440	21.9	July 11	August 21	41

<sup>&</sup>lt;sup>a</sup> Hopkins states: "Other things being equal, the variation in the time of occurrence of a given periodical event in life activity in temperate North America is at the general average rate of four days to each 1 degree of latitude, 5 degrees of longitude, and 400 feet of altitude, later northward, eastward, and upward in the spring and early summer, and the reverse in late summer and autumn" (Hopkins, p. 7, 1918).

<sup>b</sup> Approximate.

days earlier (-) or later (+) than July 3—the average date of maximum bloom during these 19 years—when the date of maximum bloom occurred in each season.

In each year excepting 1920 and 1935 when the average temperatures during April, May, and June were above the mean, the date when the maximum number of plants were in full bloom occurred earlier than the median date; when the daily temperatures were below the mean, the date when the maximum number of plants were in full bloom was later than the median. For each degree of average daily temperature above the mean, the average date of full bloom was advanced two days earlier than the median, and for each degree below the mean the date when the maximum number of plants were in full bloom was delayed two days.

Rôle of length of day in relation to the growth of timothy.-Garner and Allard (1920) showed that in many species of plants development is profoundly affected by differences in the periods of daily illumination. In timothy (Evans and Allard, 1934) late plants require longer days for the development of culms and inflorescences than early ones. Roberts and Struckmeyer (1937, p. 647) have found that timothy, like many other kinds of plants, gives different photoperiodic responses at different temperatures.

In an experiment conducted at Washington, D. C. (Evans and Allard, 1934), early timothy plants produced normal culms and inflorescences with from 12 to 12.5 hours' illumination, while very late plants required 15 hours or more for these processes. Under natural lengths of day, the first florets bloomed on plants of 19456, the earliest selection, on June 3; on plants of 6127, a selection blooming about the same time as ordinary timothy, on June 19; on 19460, the next to the latest selection, on July 22. With the daily illumination increased to 18 hours, on the plant of 6127 the first florets bloomed on June 3, and on 19460, on June 4. When grown under a sufficiently long day, therefore, the medium and the late selections bloomed as early, or very nearly as early as the selection (19456) which, when grown under natural conditions, was the earliest in the

The more rapid development of many kinds of plants at northern latitudes than at places relatively far south (Albright, 1933) is due largely to this acceleration which occurs when they are grown under the very long days of spring and summer in the far North.

PROCEDURE.—Nine F. C. selections of timothy were used, ranging with fairly uniform gradations from very early to very late. With the exception of the three latest strains, which were of Danish and Russian origin, these selections were derived from ordinary American timothy. Number 6743 heads.

\* The F. C. selections have been assigned accession numbers by the Division of Forage Crops and Diseases, Bureau of Plant Industry of the United States Department of Agriculture.

blooms, and matures at approximately the same time as ordinary timothy; numbers 19456, 19458, and 11902 are earlier, and numbers 12421, 19416. 19459, 19460, and 28023 are all later than ordinary American timothy.

The plants were sent from the Timothy Breeding Station in 1934, then located at North Ridgeville, Ohio, to the nine other stations, extending over more than 28 degrees of latitude. Since the plants had been propagated vegetatively from the original plant of each selection, variations, which would have occurred if the plants had been grown from seed, were eliminated.

The characteristics observed include: the habits of growth of the stems, whether upright, decumbent or procumbent; the lengths of the stems; the extent of development of inflorescences; and the dates when the first florets bloomed. The relative times of heading, blooming, and maturing of different selections were not greatly different (Evans, 1922). Records obtained in 1936 and 1937 do not differ greatly from those in 1935, which are presented in this

In the experiment conducted at Lexington, Kentucky, and at North Ridgeville, Ohio, the data in regard to the yields of hay were obtained at each

station from replicated broadcast plots.

HABITS OF GROWTH OF EARLY, MEDIUM, AND LATE PLANTS AT DIFFERENT LATITUDES .- The plants of any selection of timothy, especially late ones, may assume different forms at different latitudes.

At Statesville, North Carolina, at latitude 35.8 degrees, several of the early selections produced upright culms; the plant of 11902, which is a few days earlier than ordinary timothy (fig. 2A) had essentially the same appearance as plants of the same selection grown at stations farther north. On the other hand, at Statesville, plants of very late selections, as 19460 (fig. 2B), produced only short decumbent culms. This same very late selection, grown as far north as Beaverlodge, Alberta, Canada, at 55.2 degrees, produced upright culms, and the form of the plant was like that shown in figure 2C.

Early selections were found to have upright habits of growth, both in the North and in the South. Late selections had decumbent culms in the South -being increasingly decumbent as the selections were increasingly later. At the stations farthest north, on the other hand, even the latest selections had upright culms. At mid-latitudes, the plants of the very late selections had intermediate habits of growth.

Lengths of the stems.—The length to which the longest stem of each plant grew is recorded in table 3.

At the southern stations, the lengths of the culms were gradually shorter as the time of heading, blooming, and maturing of the plants became later. At mid-stations plants which bloomed and matured at approximately a medium date in general produced longer culms than either the early or the late selections. At the two most northern stations from

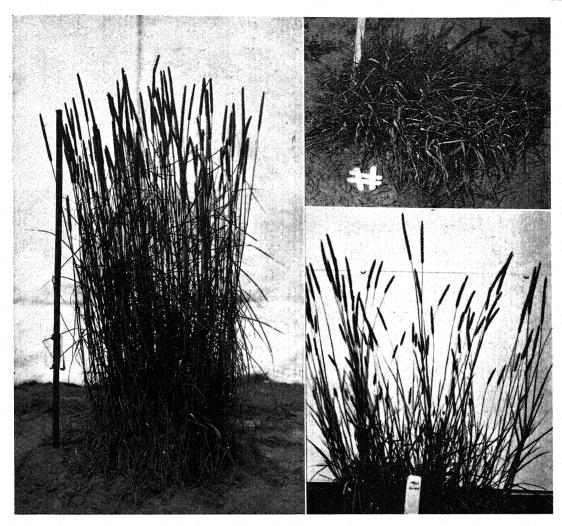


Fig. 2.—A (left). Timothy number 11902, somewhat earlier than ordinary timothy, grown at Statesville, North Carolina, at latitude 35.8.—B (upper right) and C (lower right). F. C. 19460, a very late selection of timothy.—B. Grown at Statesville, North Carolina; the plant had short, decumbent stems and no inflorescences.—C. Grown at Beaverlodge, Alberta, Canada, 55.2 north latitude; the plant produced upright culms and inflorescences.

which records for all selections were obtained, the lengths of the longest culms of the two latest were only five to six inches shorter than those maturing at a medium time.

Differences in the lengths of the stems at a southern and at a northern station are illustrated in figure 3.

Development of the inflorescences.—At Gainesville, Florida, only the earliest selection, 19456, produced inflorescences; the later ones produced only short, more or less decumbent, barren shoots. At Belle Glade, Florida, also, 3 degrees farther south than Gainesville, where all these selections were planted, number 19456 was the only one which produced culms with inflorescences. At Statesville, North Carolina, all excepting the two latest selections produced inflorescences. At Washington, D. C., at latitude 38.9 degrees and at North Ridgeville, Ohio, at 41.4 degrees, although all selections pro-

duced inflorescences, yet on the three latest ones a large proportion of the culms were barren. Farther north, as at Beaverlodge, Alberta, where the culms of all selections were upright, even the latest plants produced large numbers of inflorescences.

Time of blooming.—Table 4 shows a greater spread in the time of blooming at the southern than at the northern stations.

The season of blooming for the earlier selections progressed from south to north. On the other hand, each of the four latest selections began to bloom at some station north of the most southern one, and from there the season for blooming progressed both toward the north and also toward the south.

The data presented in the preceding paragraphs are from a series of stations extending in a general direction from southeast to northwest. Similar results were obtained in 1933 at La Jolla, California, and at North Ridgeville, Ohio, 8.6 degrees far-

ther north and 35.25 degrees farther east; numbers 19456, 19458, and 11902, all early selections, began to bloom at La Jolla on May 6, June 10, and July 9, and at North Ridgeville on June 5, June 8, and June 10, respectively. The season for blooming of selections 19458 and 11902 progressed from the northern station toward the southern one; the season progressed from the south to the north only for the earliest selection.

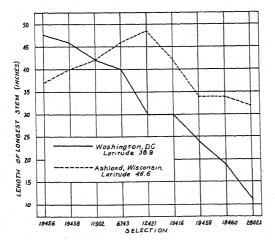


Fig. 3. Lengths of longest stems of early, medium, and late timothy plants at two latitudes. The selections are arranged in order of time of blooming, from the earliest at the left to the latest at the right.

YIELDS OF HAY AT DIFFERENT LATITUDES.—In an experiment conducted in 1933, 1934, and 1935, at Lexington, Kentucky—near the latitude of Washington—records were obtained of the yields of hay produced by two F. C. selections of timothy which, at North Ridgeville, Ohio, were about four and five days earlier, respectively, than ordinary timothy—i.e., numbers 11901 and 12468—and by two late

selections, numbers 3937 and 12368, which at North Ridgeville are about six and seven days later, respectively, than ordinary timothy.

The average yields of hay per acre at Lexington, Kentucky, are presented in table 5. The yields of both early selections were greater and of both late selections were less than the yields of ordinary timethy.

The same strains of timothy were grown at North Ridgeville in 1931, 1932, and 1933. In the latter year, ordinary timothy at North Ridgeville produced 2908 pounds per acre, compared with a yield of 1660 pounds at Lexington. The larger yield at North Ridgeville than at Lexington is apparently correlated with the tendency of the stems to grow to greater lengths, and for a larger proportion of them to produce inflorescences at northern than at southern latitudes.

If the yields of ordinary timothy are assumed to be 100, then at the northern station, the average yields of the early and of the late strains were 104 and 108, respectively; at the southern station, 138 and 76, respectively.

DISCUSSION OF THE EXPERIMENTAL RESULTS.—In order that the plants of any strain of timothy may produce culms and inflorescences, both the daily temperatures and the lengths of day must equal or exceed the minimum requirements for that strain. As long as either condition fails to meet the required minimum, the plants remain in a vegetative state. In the South, temperatures are suitable for reproductive development in early spring, but the days are too short. In the North, days become of suitable length earlier than in the South, but temperatures are too low for growth and development.

Progress of the season from one latitude to another.—The progress of the season for blooming is shown in table 4 and figure 4.

For the earliest strains of timothy, at the southern border of the timothy growing area in the north-

Table 3. Length of the longest stem of timothy plants in 1935. The selections are arranged in order from the earliest, 19456, to the latest, 28023.

				Station a	and latit	ude		
	States- ville, N. C.	Wash-ington, D. C.	N. Ridge- ville, Ohio	Guelph, Ont.	Ash- land, Wis.	Brandon, Man.	Beaver- lodge, Alta.	Fort Vermilion, Alta.
Number	35.8	38.9	41.4	43.5	46.6	49.8	55.2	58.4
	In.	In.	In.	In.	In.	In.	In.	In.
19456	. 53	48	47	45	37	42	42	(b)
19458	. 58	46	48	49	40	47	44	(b)
11902	. 40	42	49	52	42	43	39	44
6743	. 37	40	54	52	46	48	46	(b)
12421		30	53	56	48	49	49	(b)
19416	. (a)	30	40	49	42	44	45	(b)
19459		24	29	39	34	36	38	(b)
19460	. (a)	19	32	41	34	38	39	(b)
28023	. (a)	11	25	36	32	43	44	(b)

<sup>(</sup>a) No records were obtained.

<sup>(</sup>b) No plants of this number were grown.

Table 4. Dates in 1935 when the first florets bloomed on plants of timothy, at stations at different latitudes. The records of the selections are arranged from the earliest to the latest, according to their dates of blooming at Washington, D. C.

				Station	and degree	es north la	titude			
Number	Gaines- ville, Fla. 29.6	States- ville, N. C. 35.8	Wash- ington, D. C. 38.9	North Ridge- ville, Ohio 41.4	Guelph, Ont. 43.5	Ash- land, Wis. 46.6	Brandon, Man. 49.8	Saska- toon, Sask. 52.1	Beaver- lodge, Alta. 55.2	Fort Vermilion, Alta. 58.4
19456	April 7 (a)	May 12	June 3	June 16	June 29	June 24	July 2	(c)	July 13	(c)
19458	(b) `´	May 30	June 8	June 20	June 28	June 30	July 1	(c)	July 11	(c)
11902	(b)	June 6	June 13	June 25	July 2	July 5	July 5	July 7	July 13	July 14
6743	(b)	June 15	June 19	June 30	July 4	July 10	July 7	(c)	July 16	(c)
12421	(b)	June~30	July 1	July 5	July 7	July 13	July 10	(c)	July 13	(c)
19416	(b)	August 1	July 5	July 7	July 8	July 12	July 9	(c)	July 18	(c)
19459	(b)	(b)	July 17	July 14	July 12	July 17	July 13	(c)	July 24	(c)
19460	(b)	(b)	August 3	July 14	July 9	July 19	July 14	(c)	July 21	(c)
28023	(b)	(b)	August 12	July 18	July 15	July 20	July 13	(c)	July 21	(c)

<sup>(</sup>a) The date and place where each selection first bloomed is indicated by italics.

(b) No florets bloomed.

ern hemisphere, the days are of sufficient length for the development of culms and inflorescences when or soon after the weather has become warm enough for spring growth. For these early strains, the season for heading, blooming, and maturing progresses northward at a gradually accelerated rate as the season advances.

Table 5. Average yields of hay in 1933, 1934, and 1935 at Lexington, Kentucky, of early, medium, and late strains of timothy.

Str	ain Yie	ld per	acre
		Lb.	
Early	11901 12468	2356 2337	
	Average		2346
Medium Late	Ordinary timothy 3937 (Huron timothy) 12368	1299 1275	1699
	Average	•	1287

<sup>\*</sup> Records obtained by Professor E. N. Fergus of the Kentucky Agricultural Experiment Station.

For a medium maturing strain, as 6743, the season for heading, blooming, and maturing, is at first delayed, after temperatures are suitable, until the days become of sufficient length, but eventually begins at the southern border of the timothy area, progressing northward, at first slowly then at a constantly accelerated rate.

For the latest strains of timothy, the time for heading, blooming, and maturing is delayed for an even longer time. The plants begin to bloom, not at the southern border of the timothy growing area, but at a latitude where suitable temperatures, and suitable lengths of day first occur. At or near the place where the minimum requirements of both conditions are first fulfilled, the plants of any very late strain of timothy begin to bloom, and the season for blooming then progresses in both directions—toward the north as the temperatures become higher, toward the south as the required lengths of day occur.

Under natural conditions, where timothy is adapted, possibly the season for blooming may nowhere progress from north to south, as in this experiment. In the southern part of the timothy area

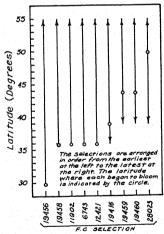


Fig. 4. Progress of the season in 1935 for blooming of different selections of timothy.

the very late strains do not reproduce themselves through seeds. Even for some distance north of the southern limits where they produce culms with inflorescences, the florets bloom late, and little or no seed matures. Consequently, if introduced there,

<sup>(</sup>c) No plant of this number was grown.

they would gradually be replaced by biotypes better adapted to such conditions.

Adaptation of early, medium, and late varieties at different latitudes.—The degree of adaptation of any variety of timothy at any latitude depends very largely upon how well the characteristics of the plants, especially their adaptations to days of different lengths, are suited to the conditions.

In the North, either early or late varieties of timothy may be grown, since the days are of sufficient length for the development of plants of either type. The choice of a variety would depend largely upon farm management practices.

In the South, because of the short days of early spring, the time when late varieties begin to produce culms and inflorescences is delayed until so late in the season that the culms do not attain full length, and the yields of hay are small. The choice of varieties for the southern part of the timothy growing area, consequently, is necessarily restricted to those which are early.

#### SUMMARY

For the purpose of obtaining information in regard to certain phases of the growing habits of timothy as affected by latitude, plants of nine selections, ranging by fairly uniform gradations from very early to very late, were grown at each one of ten stations located at intervals extending from Gainesville, Florida, to Fort Vermilion, Alberta, Canada.

In the South, selections which are progressively later had progressively shorter stems; the stems of the latest selections are more or less procumbent there. In the North, the stems of the later selections grew to as great, or even greater lengths than those of the early selections.

The time of heading, blooming, and maturing is determined largely by the temperatures and by the lengths of day. Late selections require longer days than early ones for the production of culms and inflorescences.

For the earliest selections, the season for blooming progressed from south to north. For the latest selections, blooming occurred first at some mid-latitude; from this latitude, the season for blooming progressed both toward the North as the temperatures became suitable for growth and toward the South as the number of hours of daily illumination gradually increased.

At a northern station, both early and late selections produced relatively large yields. At a southern station, late selections produced smaller yields of hay than early selections, which are better adapted to the relatively short days occurring there.

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## RELATIONS BETWEEN TISSUE ORGANIZATION AND VEIN DISTRIBUTION IN DICOTYLEDON LEAVES <sup>1</sup>

Robert B. Wylie

This paper deals with certain relations between leaf structure and the distribution of minor veins in the blade. In connection with work on the wound responses of foliage leaves the writer built up several series of slides which were equally favorable for the study of normal leaf tissues. It seemed from reviewing these preparations that vein spacing was often related to mesophyll organization. A careful analysis of leaves of three series of plants seemed to confirm this generalization and revealed a fairly close correlation between the photosynthetic and conductive tissue of the blade in these species.

Several investigations have dealt quantitatively with the development of the minor venation in the leaf blade. Zalenski (1902), using leaves cleared in chloral hydrate and mounted in glycerin, called attention to variations in total length of vein per unit area in plants of given species even in similar habitats. This led to his later paper (1904), which is discussed at length by Maximov. He demonstrated a wide range in the structure of leaves distributed vertically along a single stem. On shrubby or herbaceous plants of moderate size he found leaves of higher insertion successively more xeromorphic with progressively greater vein length per unit area than those lower on the same stem. While this feature was associated with several other structural differences in the leaves investigated, he considered these vein specializations as most closely correlated with cell size in the different leaves.

Schuster (1908) surveyed a considerable number of plants, giving attention to differences between sun and shade leaves. With few exceptions, shade leaves were larger than sun leaves of the same species, but the shaded leaves had reduced venation, in terms of total vein length per unit area of blade, compared with those in the sun.

Benedict (1915) made an intensive vein study of a few plants but concerned himself particularly with Vitis vulpina. He held that vein spacing (which he termed the vein-islet) for leaves of this species was correlated closely with the plant's age, diminishing regularly through successive years after the launching of a new plant from an embryo started by fertilization. His records were made photographically by transmitted light, using untreated living leaves, which method probably failed to record some of the lesser veinlets. While he noted the earlier work of Zalenski (1902), he does not include the very important paper by that writer published in 1904.

Strain (1933) recently made a study of the types of vascular elements in the vein endings of a large number of species, by clearing whole leaves, cotyledons, and petals. He finds type patterns of tracheal organization rather than variations due to environ-

<sup>1</sup> Received for publication January 16, 1939.

ment. Vein endings of sun leaves and shade leaves did not differ within the species, either in arrangement or shape.

MATERIAL AND METHODS.—The leaves, cut into small rectangles, were mostly killed and preserved in 3 per cent formalin, imbedded in paraffin, and sectioned (12 µ thick) in both transverse and paradermal planes. Leaves from trees, shrubs, and herbaceous plants were included in collections from a number of widely separated regions. In this paper dicotyledon leaves of three series are discussed: (1) Iowa woody plants (table 1), (2) Iowa herbaceous plants (table 1), and (3) a group of broad-leaved woody plants, mostly evergreens, from the Pacific coast regions, including plants from western Washington, Oregon, and southern California (table 2). Sufficient numbers of species were included to constitute a representative sampling for each of the three groups surveyed.

In all instances sun leaves were selected, or in the case of shade plants, those with average illumination. While both trees and shrubs are included in the woody series, all collections were uniformly taken at such height as to be within reach from the ground. While leaves of this level may differ from those higher on the same plants, comparisons between taller plants would be fairer if leaves of like exposure and of corresponding height of insertion were used. While this survey includes several plants not native to Iowa, only such were taken as have long and regularly grown in this state. The Pacific coast species are all native and nearly all of them evergreen.

In this study the tissues of the blade, between veins, have been resolved, as accurately as possible, into vertical and horizontal elements. The epidermal covering involves the only continuous cell layers of the blade. While protective in some degree, due primarily to the cuticle, these layers sustain a basic relation to the other tissues through their mechanical and conductive functions. The upper and lower epidermal layers together constitute about one-fifth of the leaf's volume in each of the three groups. This ratio would be considerably increased in leaves having multiple epidermis, but these are not included in this study excepting only Berberis nervosa, which has a subdermal mechanical layer of cells 15  $\mu$  thick.

The spongy mesophyll, like the epidermis, is primarily a zone of horizontal integrity and is generally an intermediary tissue between the more specialized photosynthetic cells and adjacent minor veins. This tissue can be correctly interpreted only from sections parallel with the epidermis, as evidenced by its inchoate appearance in transverse sections of the leaf. It carries some of the chlorophyll and usually has outstretched arms from each cell

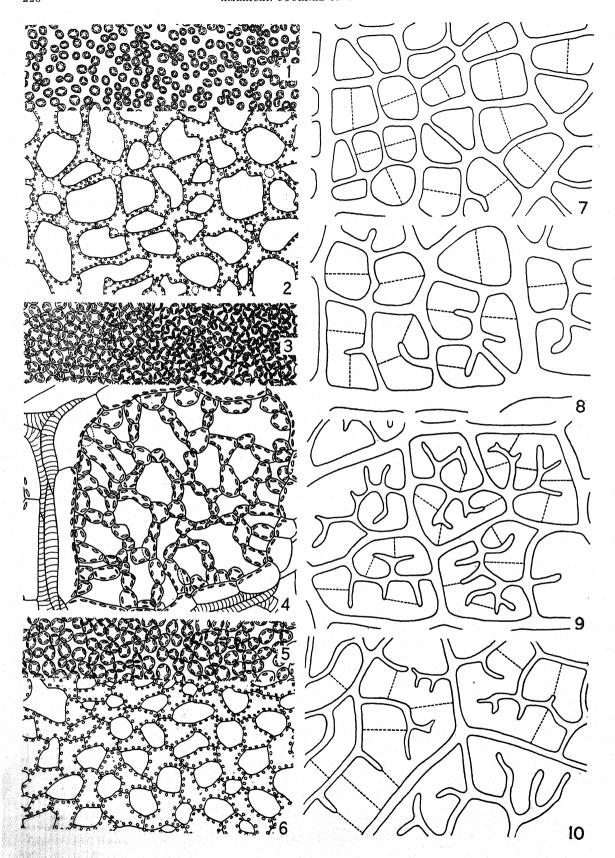


TABLE 1a. Iowa woody plants.  $r = 0.735 \pm .0597$ 

(Measurements in microns)	Up. ep.		Spg. par.	Low. ep.		Total thick.	Int. vas.	%ª SEE	,
Juglans nigra L	15.8	56.0	106.5	16.0	16.5	194.3	185.0	71.2	29.0
Sambucus canadensis L	21.0	38.5	81.0	17.5	13.0	158.0	174.0	75.6	24.0
Parthenocissus quinquefolia Planch	18.6	63.2	69.8	14.0	15.4	165.6	165.0	61.8	38.0
Aristolochia durior Hill	14.0	34.0	40.0	7.0	11.0	95.0	161.0	64.2	36.0
Catalpa speciosa Warder	15.0	57.0	56.0	15.0	14.0	143.0	144.0	60.1	40.0
Acer saccharinum L	12.4	36.6	26.4	11.4	10.2	86.8	142.8	57.9	42.0
Cercis canadensis L	24.0	36.0	45.0	17.0	12.0	122.0	129.0	70.4	30.0
Vitis vulpina L	15.4	65.0	56.8	12.7	16.6	149.9	123.7	56.6	43.0
Populus grandidentata Michx	12.4	81.4	34.0	9.8	9.7	137.6	111.0	40.8	59.0
Pyrus ioensis Brit	23.5	58.5	45.0	14.1	11.0	141.1	110.7	58.5	41.0
Syringa vulgaris L	26.0	174.0	94.0	19.0	14.3	313.0	105.0	44.4	56.0
Crataegus sp	21.0	35.0	39.0	9.0	0.0	104.0	101.0	66.4	34.0
Tilia americana L		36.0	41.0	17.0	13.0	113.0	100.0	68.2	32.0
Quercus velutina Lam		72.8	63.0	17.2	12.0	184.6	86.9	60.6	39.0
Rhus glabra L	15.0	103.0	51.3	11.3	11.3	180.6	82.5	42.9	57.0
Quercus macrocarpa Michx		63.0	42.8	10.0	7.8	136.2	82.0	53.7	46.0
Platanus occidentalis L	21:8	82.2	42.0	18.4	10.6	164.4	74.0	49.9	50.0
Ulmus americana L		87.0	44.6	13.8	12.0	167.4	69.0	47.9	52.0
Populus deltoides Marsh	12.6	119.1	30.0	12.1	10.0	173.8	67.7	31.4	69.0
Populus canadensis Moench		114.4	24.0	12.0	8.6	166.1	65.0	31.1	69.0
Ailanthus glandulosa Desk	19.9	83.0	55.7	13.9	8.5	172.3	61.0	51.9	48.0
Robinia Pseudo-Acacia L		58.6	28.0	14.0	8.0	117.2	60.0	49.9	50.0
Mean	19.0	35.0	51.0	13.8	10.9	152.5	112.1	55.4	44.6

which unite with similar extensions from adjacent cells, leaving large intercellular spaces (fig. 2, 4, 6). Its porosity (in the dorsiventral blade) permits ready diffusion of gases to and from the palisade. A high proportion of spongy mesophyll because of its lateral connections between cells simplifies conduction between veins.

The palisade parenchyma offers sharp contrast to the epidermis and sponge (fig. 1, 3, 5). Its cylindric or prismatic cells, elongated at right angles to the leaf surface, have limited lateral contacts and thus present a much greater surface against intercellular space involving at times a high transpiration rate (Turrell, 1936). The palisade type of mesophyll complicates conduction problems and involves both increased amounts and indirect routes of transfer between major photosynthetic cells and conductive tissues and thus becomes a factor in vein distribution.

Various measurements of the leaves surveyed are summarized in tables 1 and 2. While several of the columns need no explanation, it should be recalled that the distance between veins or the intervascular interval (Int. vas.) is based upon measurements from xylem to xylem. The width of the border parenchyma (Bor. par.), which shares in some degree in the conductive work, is also given in all cases. Column SEE is the total thickness of the epidermal layers together with that of the spongy mesophyll, if present. The thickness of blade (Total thick.) was measured between veins. It should be noted that each number in the tables represents an average value based upon ten to twenty or more measurements of the item in question. The final columns at the right show the percentile distribution of blade thickness between the cell layers of greater horizontal integrity, the sponge and epidermis (SEE), and that given over to palisade (Pal.) with its vertically elongated cells. Since the different tissues were measured independently, the sum of the numbers approximates 100 per cent but may vary slightly from unity. In these tables the species are arranged with respect to their intervascular intervals, the one with widest spacing between veins is placed first and others in order, the last having least distance between vascular strands. Thickness of cuticle is included in epidermal measurements and in total thickness of blade.

Discussion.—Due to lack of uniformity of vein size and pattern in different leaves, the term intervascular-interval has been used to denote the mean maximum distance between veins (xylem to xylem) as seen and measured, using paradermal sections. It should be noted that the minor venation is closely integrated, whatever the pattern, and constitutes a plexus of channels that extends far and wide in the

Fig. 1-10. All drawings were made from paradermal sections, cut parallel to the leaf surface.—Fig. 1-2. Juglans nigra, palisade and spongy mesophyll.—Fig. 3-4. Querous agrifolia, palisade and spongy mesophyll.—Fig. 5-6. Vitis vulpina, palisade and spongy mesophyll.—Fig. 7. Vein pattern of Tilia americana.—Fig. 8. Vein pattern of Querous agrifolia.—Fig. 9. Vein pattern of Populus deltoides.—Fig. 10. Vein pattern of Ricinus communis.

Table 1b. Iowa herbaceous plants.  $r = 0.648 \pm .0803$ 

										_
(Measurements in microns)	Up.		Spg. par.	Low. ep.		Total thick.	Int. vas.	%ª SEE	% Pal.	
Plantago major L	19.5	0.0	140.0	15.0	16.2	174.5	262.0	100.0	0.0	
Eupatorium urticaefolium Richard	14.0	25.0	30.0	9.0	10.0	78.0	255.0	67.9	32.0	
Sircaea lutetiana L	17.0	24.0	41.0	12.0	11.5	94.0	201.0	74.5	26.0	
ilphium perfoliatum L	16.0	88.0	122.0	15.0	23.0	241.0	181.0	63.4	37.0	
icia faba L	24.6	68.0	130.0	16.5	13.8	239.1	177.0	71.5	28.0	
Chenopodium album L	23.1	64.5	89.1	15.0	23.8	191.7	157.0	66.1	33.6	
xybaphus nyctagineus (Michx.) Sweet	20.9	106.0	94.0	17.5	22.4	238.4	155.0	55.5	44.0	
retium minus Bernh	17.0	44.1	49.8	10.5	10.9	121.4	149.0	63.8	36.0	
maranthus retroflexus L	22.3	39.4	106.5	17.4	42.7	185.6	147.5	78.5	21.0	
ntennaria sp	20.6	51.1	68.0	14.6	16.0	154.3	146.0	66.9	33.0	
ycopersicon esculentum Mill	16.0	56.2	65.0	18.0	11.0	155.2	141.0	63.9	36.0	
ropaeolum majus L	20.1	68.0	58.0	21.1	10.0	167.2	140.0	59.4	41.0	
icotiana alata var. grandiflora Comes	22.0	104.0	121.7	20.3	12.0	268.0	136.0	61.2	39.0	
olvgonum pennsylvanicum L	20.8	79.4	50.7	17.0	15.4	167.9	121.0	52.7	47.0	
olanum tuberosum L	14.5	59.0	66.0	11.5	17.0	151.0	120.0	60.9	39.0	
mbrosia trifida L	11.6	59.0	39.0	10.0	10.3	119.6	120.0	50.5	49.0	
Ascelpias syriaca L	26.0	54.0	114.4	13.4	13.0	207.8	117.0	74.1	26.0	
erbascum Thapsus L	16.0	43.0	58.0	11.5	12.8	128.5	92.0	66.2	33.0	
Ambrosia artemisiifolia L	11.8	65.5	41.4	11.8	11.8	130.5	98.0	49.6	50.0	
rifolium pratense L	21.0	36.0	57.0	21.0	7.0	135.0	80.0	73.4	27.0	
olidago rigida L	17.8		0.0	13.4	12.0	183.0	76.0	17.0	83.0	
va xanthifolia Nutt	19.2		0.0	15.8	10.8	83.8	75.0	41.8	58.0	
Ricinus communis L	17.5	79.4	74.5	14.1	9.8	185.5	64.0	57.0	43.0	
Abutilon Theophrasti Medic	12.0	44.6	21.6	8.0	9.0	86.2	55.0	48.3	52.0	
Mean	18.4	60.7	68.2	13.8	14.7	162.6	136.0	61.7	38.6	-

<sup>\*</sup> SEE is the combined thickness of spongy mesophyll and epidermal layers.

blade except as interrupted by larger veins. Some leaves have definite polygonal islets (fig. 7), but in the majority of species the intrusion of vascular branches, which are often repeatedly divided, results in a vein system which has little resemblance to the net-like type (fig. 8–10). An average of the measurements across carefully selected major intervals gives a fairly accurate interpretation of the vascular distribution of a given leaf.

The border parenchyma as a sheath about minor veins shares in the transfer of materials and is intermediary between mesophyll and vein. Dorsiventral extensions of this bundle investment increase the number of cell contacts and may even reach one or both of the epidermal layers. The importance of the border parenchyma has not been adequately stressed by botanists. However, owing to its relative uniformity, it is not given special consideration in this paper, and its radial thickness is included in the intervascular-interval.

By way of factual summary it may be stated that this survey includes leaves from plants of wide geographic and taxonomic distribution, ranging from soft, mesophytic, shade plants to sclerophylls of desert areas in California. Among them are herbs, shrubs, and trees of great diversity having leaves of wide range in size, form, thickness, texture, and longevity. While the epidermal layers vary less, the mesophyll organization ranges from predominant sponge to pure palisade. The venation of these three

groups of dicotyledons offers similar diversity of pattern and distribution with no type peculiarly distinctive of any habit or habitat. The arrangement in any of the groups may be that of the simple islettype or may involve modified patterns having elaborately branched minor veins. In spite of the wide fluctuations in all parts and tissues among the leaves of the 66 species covered in this survey, an attempt has been made to correlate blade structure and vein distribution. While other factors obviously are involved, measurements reveal for each of the groups a significant relation between the relative amounts of vertical and horizontal tissues in the blade when compared with the intervals between veins of the same leaves. Variations in the orientation of the mesophyll cells are likely to be associated with readjustments of the intervascular-interval. Increasing proportions of palisade tend, other things being equal, to force veins nearer together, while greater relative amounts of spongy mesophyll permit wider separation of vascular channels.

The broad leaved dicotyledons of the Pacific regions for the 20 species surveyed had nearly equal amounts of paradermal tissues and palisade mesophyll, with a mean intervascular interval of  $140.2~\mu$  (table 2). For this group the coefficient of correlation between blade organization and vein distribution was .684 with a probable error of  $\pm 0.0804$ . The Iowa herbaceous plants, represented by 24 species, had relatively more paradermal tissues in the

Table 2. Pacific Coast woody plants  $r = 0.684 \pm .0804$ 

•									
	Up.		Spg. mes.			Total thick.	Int. vas.	%ª SEE	% Pal.
Berberis nervosa Pursh	31.0	51.0	167.0	14.0	16.0	263.0	337.0	81.0	19.0
Gaultheria shallon Pursh Washington	25.0	74.0	133.0	18.0	11.0	250.0	316.0	70.0	30.0
Chimaphila umbellata Nutt Washington	30.0	50.0	170.0	25.0	13.0	275.0	274.0	82.0	18.0
Viburnum ellipticum HookOregon	22.0	71.0	119.0	20.0	21.0	232.0	206.0	70.0	30.0
Arbutus menziesii Pursh Washington	26.0	137.0	102.0	18.0	10.0	283.0	171.0	52.0	48.0
Photinia arbutifolia LindlCalifornia	38.0	129.0	141.0	26.0	19.0	334.0	146.0	61.4	39.0
Berberis aquifolium PurshOregon	23.0	43.0	53.0	17.0	17.0	136.0	140.0	68.0	32.0
Arctostaphylos columbiana PiperOregon	21.0	61.0	44.0	13.0	13.0	139.0	119.0	56.0	45.0
Amelanchier alnifolia NuttOregon	21.0	351.0	0.0	21.0	14.0	393.0	125.0	11.0	89.0
Rhamnus crocea NuttCalifornia	21.5	68.0	125.0	16.0	18.3	230.5	118.0	70.5	30.0
Quercus agrifolia Nee	18.6	96.6	88.0	13.3	11.5	216.5	118.0	55.4	45.0
Salix scouleriana Barr	19.0	68.0	39.0	11.0	11.0	137.0	95.0	50.0	50.0
Lonicera hispidula Dougl California	33.0	64.0	49.0	17.0	11.0	163.0	95.0	60.7	39.0
Quercus dumosa Nutt	22.0	201.4	60.0	18.2	12.0	201.6	90.0	33.3	77.0
Quercus Douglasii H. and ACalifornia	25.7	204.0	40.0	12.3	15.0	282.0	86.0	27.6	72.0
Rhamnus purshiana DC	29.0	67.0	20.0	12.0	11.0	128.0	85.0	48.0	52.0
Rhus laurina Nutt	38.0	462.0	0.0	41.0	12.0	541.0	76.0	14.6	85.0
Salvia mellifera GreeneCalifornia	35.0	77.0	34.0	8.0	12.0	154.0	75.0	50.1	50.0
Rhamnus californica EschCalifornia	36.0	121.0	25.0	15.0	9.2	197.0	67.0	38.6	61.0
Rhus integrifolia NuttCalifornia	33.0	322.0	76.0	35.0	14.4	467.0	66.0	30.9	69.0
Mean	26.6	135.9	74.2	18.5	13.5	251.1	140.2	51.6	49.0

<sup>&</sup>lt;sup>a</sup> SEE is the combined thickness of spongy mesophyll and epidermal layers.

blade (61 per cent) and had a much wider vein separation with a mean intervascular interval of 136.0 µ (table 1). For these the coefficient of correlation was .648 and the probable error  $\pm 0.0803$ . The Iowa woody plants, 22 species, constitute a somewhat intermediate group, with approximately 55 per cent of the blade tissues horizontally integrated, and had a mean intervascular interval of 112.1  $\mu$  (table 1). For this group the coefficient of correlation between tissue arrangement and vein separation was .735, with a probable error of  $\pm 0.0597$ . Smaller groups of species from other areas, similarly surveyed, gave relatively higher correlations between blade structure and vein separation, but the results were not included in this paper because of limited samplings. It seems, however, that conclusions based upon 66 species, representing three groups of such diverse habit and habitat might be considered significant.

That other and important factors enter into the problem of minor vein distribution is obvious. Total leaf thickness, cell size, compactness of tissues, relative epidermal thickness, and possibly the nature of the border parenchyma should influence internal tissue relations. Most of these, however, would operate through their effects upon conduction to and from veins. For example, many leaves with large amount of palisade have increased lateral contacts between these cells, thus simplifying paradermal conduction. An analysis, to be complete, should measure the degree or amount of lateral integration between cells of all blade tissues and should evaluate the effects of added cell layers as found in thicker leaves. The well known differences between different parts of

the same blade should govern the selection of material. The thickness of the cuticle, which is considerable in broad leaved evergreens, has in this study been included in epidermal thickness though it is obviously not helpful in lateral conduction. Any considerable differences between the paradermal and vertical dimensions of the border parenchyma likewise should be taken into account. Obviously, cell size has an important bearing on conduction problems, as noted by Zalenski (1904), and illustrated by Tellefsen (1922).

It seems probable that the vascular system of a leaf develops conservatively, to be extended within limits in relation to conduction demands foreshadowed by conditions during later leaf development. The differences between the mesophyll of sun and shade leaves on the same plant are well known, but it is not so generally recognized that the venation is correspondingly modified, as has been shown by Schuster (1908) and others. This suggests that during the development of a leaf its tissues follow a more or less specific pattern but are modified in some degree by local conditions during ontogeny. The deepening shade in the developing crown of a tree introduces gradual differences in the amount of light reaching interior leaves. The cells primarily concerned with the development of the minor veins retain longest a capacity for normal cell division. Mounts (1932) has shown for Vitis vulpina that the cell layers giving rise to the minor veins are the last to mature. This prolonged capacity for cell division in this zone aids the leaf in modifying its vein pattern to the type of mesophyll being developed in that particular leaf. The dominant hereditary pattern and the probability that differences may be impressed upon leaf rudiments through bud position and exposure during early stages need not hinder subsequent adjustments of veins and tissues during later development under somewhat different situation.

The relatively long time required for growth of a leaf favors some adjustment of the given organ in relation to its particular location. If the period of development were too brief, days rather than weeks, a number of successive cloudy days at a critical period might give the leaf a pattern less suited to its average adult exposure. A longer period of plasticity permits closer approach to the type fitted to the leaf's immediate environment. It may be unfortunate for some deciduous trees of the upper Mississippi valley that their leaf pattern is worked out so early in the season, at a time of cooler weather and more frequent rains and before the increased and prolonged transpiration demands of the summer have developed.

The palisade, whatever its advantages, involves problems relating to material transfer. As the center of the leaf's activity, its extensive surface promotes transpiration which forces water transport, and the photosynthate must also be carried out. While the elongation of its cells favors vertical (lengthwise) movement of materials within these cells, their limited lateral contacts force endwise entrance and exit through terminal walls that are restricted in area compared with the volume of these long cells. This not only lengthens the total distance for the transfer of increased loads but forces a right angle route, first downward to the spongy mesophyll, or the plane of the minor veins, then laterally to the border parenchyma.

The upper epidermis may help with conduction to or from the adjacent palisade. This covering layer has closely integrated cells with lateral walls usually pitted, thus favoring movement of materials in the horizontal plane. Its vascular connections are aided by dorsal ribs from some of the lesser veins. But the spongy mesophyll, when present, is the chief intermediary between palisade and vein. The junction plane shows the lower ends of the palisade cells closely grouped and connected with the sponge. The lateral unity of the spongy mesophyll and the fact that with rare exceptions the minor veins lie in this zone favors lateral movement to or from vascular strands. Even in the leaves which seem to be all palisade, paradermal sections often reveal a thin layer of closely connected cells in the plane of the veins (fig. 4). It seems fairly clear that the palisade/ sponge ratio is related through conduction problems to the distribution of veins in the blade.

The intervascular-interval, carefully measured from paradermal sections, seems a useful criterion in the interpretation of leaf organization. While there may be a considerable personal element in the evaluation of the more complex vein patterns, it stresses vein-spacing or, more accurately, the width

of tissue associated with conductive channels, rather than total length of veins (of all categories) per sample area. Since veins are seldom in the form of simple meshes, the term "vein-islet" is difficult in general application. Through a study of the intervascular-interval the blade, regardless of vein pattern, is resolved into narrow bands of chlorenchyma about the minor veins.

The proximity of veins reveals the limited distance through which conduction may take place from cell to cell in sufficient amount for the needs of an active leaf. The average distance between the minor veins for the 66 species included in this survey is about 130  $\mu$ . Since this is twice the maximum conduction distance, measured in the paradermal plane, the average width of tissue bordering the veins is but 65 μ. This distance is increased of course for cells that lie above or below the plane of the minor veins which are usually embedded in the spongy mesophyll. The functional unit of the foliage leaf is a limited width of tissue, mainly chlorophyll bearing, associated with each minor vein, and there exists in general a close relation between mesophyll organization and vein distribution.

#### SUMMARY

An analysis of leaf structure for three groups of dicotyledons, including 22 Iowa herbaceous plants, 24 Iowa woody plants, and 20 Pacific coast woody plants, shows a significant correlation between mesophyll organization and vein distribution.

When the tissues of the blade are resolved into two categories—(1) those of horizontal trend with cells closely united laterally (epidermis and spongy mesophyll), and (2) those of vertical elongation with limited lateral contacts (palisade)—the ratio between these two somewhat antagonistic systems is related to vein distribution. Increased amounts of palisade tend to force veins nearer together while larger proportions of spongy mesophyll favor their wider separation.

The working units of the foliage leaf are narrow bands of tissue associated with the minor veins, regardless of their arrangement. For the 66 species surveyed the mean vein separation, or intervascular-interval, was 130 microns, which is twice the conduction distance in the midplane. This suggests that problems of transfer from cell to cell restrict mesophyll to proximity to vascular supply. All tissue arrangements that further or retard conduction between living cells and veins become factors in vein separation.

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## POLLEN ANALYSIS OF A BOG IN NORTHERN IDAHO 1

Henry P. Hansen

This study is one of several concerned with postglacial forest succession in the Pacific Northwest as interpreted from pollen analysis. The author eventually expects to have completed similar studies of bogs located within most of the climax formation areas in Washington, Idaho, and parts of Oregon. The postglacial forest succession for the entire region will be correlated from these data, and conclusions drawn as to climatic trends. The bog of this study lies at an elevation of about 2,000 feet, in T. 61 N., R. 5 W., Sect. 34, on the Priest River, Idaho Quadrangle. This region was glaciated by ice of Wisconsin age, which extended as far south as Spokane (Flint, 1937). The bog has been developed in a kettle in a small, outwash-filled valley near Kalispel Creek, a few miles to the west of Priest Lake. The total area of the bog is about fifty acres, with an open lake (Hagen L.) of several acres still in existence near the south end. Near the shore of the lake a floating plant hydrosere exists, characterized chiefly by the yellow pond lily (Nymphaea polysepala). A Sphagnum moss mat surrounds the lake, which supports a heavy growth of cranberry (Vaccinium oxycoccus) and bog laurel (Kalmia polifolia). On the outer edge of the Sphagnum mat is a sharply delimited zone of young western white pine (Pinus monticola), succeeded by zones of spiraea (Spiraea menziesii), sedge (Carex spp.), and alder (Alnus sinuata). The latter grades into the forest on higher adjacent ground.

The depth of the peat in the area of sampling is 12 meters, with a thin layer of volcanic ash at 9 meters. The ash may be correlated with that found in bogs near Spokane (Hansen, 1939), and possibly with that in bogs in the Puget Sound region (Hansen, 1938; Rigg and Richardson, 1938). The lower three meters are composed of gray-brown sedimentary peat which contains a few gastropod shells. The sedimentary peat grades upward into sphagnum peat. Sphagnum moss leaves make their first appearance at 9 meters and gradually increase in abundance. The upper meter consists of shrubby peat, composed of ericaceous shrubs and Sphagnum. Peat samples were taken south of the lake at half meter intervals with a Hiller borer. For study, the peat was deflocculated by boiling in a weak solution of potassium hydrate, washed several times, centri-

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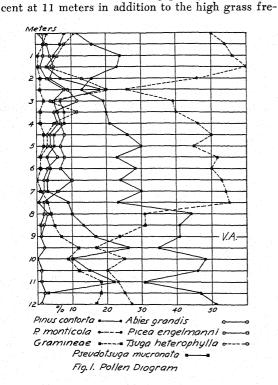
fuged, stained with gentian violet, and mounted in glycerin jelly. From 300 to 500 pollens were identified from each level. There seems to be no difficulty involved in distinguishing the pollens of pine, spruce, and fir in the Pacific Northwest. Identification may readily be based on the total size of the pollen, position of the air sacs on the cell, relative size of the sacs and cell, and the shape of the cell.

Pollens of certain species which are of no significance as indicators of forest succession usually occur in each level. Many of these species grow on or near the bog. The pollens of these species may be present in large numbers, and thus represent a high percentage of the total. In computing percentages for the curves, it seems best to let the pollens of significant species represent 100 per cent. The numbers of non-significant pollens are computed as a percentage of the total significant pollens. By this method, the relative abundance of the non-significant pollens is shown, and the curves of the significant pollens are probably more valid.

FORESTS IN ADJACENT AREAS.—The bog is situated within the Canadian Life zone as defined by Merriam (1898). On Thornwaite's map of the climatic provinces of North America, this area is also designated as having a sub-humid, microthermal climate, with adequate precipitation at all seasons (Thornwaite, 1931). Forest type maps indicate this region as largely covered with a white pine forest type (Larsen, 1930). Although white pine is often the most abundant and may occur in almost pure stands, the climax association is composed of western red cedar (Thuja plicata), western hemlock (Tsuga heterophylla), and lowland white fir (Abies grandis) (Hubermann, 1935). White pine is a subclimax species which persists as a result of fires and lumbering, which destroy the forest and prevents it from reaching its culmination (Larsen, 1929). If undisturbed for a prolonged period, the climax forest assumes the dominant rôle, and white pine persists as older individuals, scattered throughout the stand. On the dryer and exposed sites, Douglas fir (Pseudotsuga mucronata) and western larch (Larix occidentalis) thrive, while western yellow pine (Pinus ponderosa) may frequently be found. The poorer sites are occupied largely by lodgepole pine (Pinus contorta). Subalpine fir (Abies lasiocarpa) and Engelmann spruce (Picea engelmanni) inhabit the cooler and damper sites near the upper edge and above the white pine type. The mean annual precipitation in the area occupied by the hemlock-cedar-white fir association ranges from 27 to 44 inches (Larsen, 1930).

Forest succession.—The pioneer postglacial forests to invade areas adjacent to the bog consisted chiefly of lodgepole and white pines, with percentages of 51 and 27, respectively, in the lowest level (fig. 1). This would seem to indicate an initial cool period of medium dryness. The frequency of 17 per cent for grass at the same level, and of 18 per cent at 11½ and 11 meters indicates its presence in abundance. The sharp increase of Douglas fir to 22 per

cent and the decrease of lodgepole pine to 31 per



quencies may indicate a warmer and perhaps somewhat dryer climate, which is recorded to the 91/2 meter level. This period is probably to be correlated with an early warm and dry period as indicated by pollen analysis of a bog near Spokane (Hansen, 1939). Here there is a heavy influx of grass, chenopods, and composites, marking the extension of the treeless Arid Transition and Upper Sonoran Life zones. This warm and possibly dry period apparently terminated about the time of the deposition of the volcanic ash in both areas. Grass decreases from 9½ meters and maintains low frequencies throughout the rest of the spectrum (fig. 1). Lodgepole pine was likewise a pioneer species in postglacial forest succession near Spokane and in the Puget Sound region (Hansen, 1938).

A period of increasing coolness and moisture is perhaps marked by the increase in both white pine and lodgepole pine from the  $9\frac{1}{2}$  to 8 meter level. At this point lodgepole sharply decreases from 44 to 23 per cent, while white pine increases from 31 to 55 per cent at the  $7\frac{1}{2}$  meter level. This is the first time that white pine shows a higher frequency than the former, and it maintains its dominance to the present time. With the exception of this reversal of dominance, however, both are closely correlated throughout the spectrum (fig. 1).

In general both white and lodgepole pines show gradual decreases from 71/2 to 21/2 meters, at which point they have frequencies of 13 and 26 per cent, respectively, the lowest for both since the deposition of the ash. During this period, Douglas fir, western hemlock, and white fir slowly increase. The latter reaches its highest frequency of 12 per cent at 3 meters, while the former two record 18 and 20 per cent, respectively, at 21/2 meters. This period evidently represents a trend toward a climax development, which was suddenly interrupted at the time represented by the  $2\frac{1}{2}$  meter level, probably by fire. White pine again rapidly increases to 60 per cent at 11/2 meters, while lodgepole pine increases less rapidly. Another trend toward climax development occurs from this point to the surface as indicated by the decrease in white and lodgepole pines and the increase in Douglas fir and hemlock. The appearance of western red cedar pollens at  $3\frac{1}{2}$  meters is to be noted as further evidence of a recent climax trend (table 1). The history of white pine as a subclimax species in postglacial forest succession is similar to that of Douglas fir in the Puget Sound region. Here Douglas fir is subclimax to the cedarhemlock climax, and it likewise has been able to maintain its dominance during postglacial time (Hansen, 1938).

Engelmann spruce maintains frequencies of more than 5 per cent from 7 to 2 meters, while subalpine fir is represented by low frequencies throughout most of the spectrum (table 1). Traces of western yellow pine also occur in many of the levels. Alder, maple, birch, and willow pollens are abundant in the lower levels, but as previously explained, their numbers were not used in computing the percentages of the significant pollens. Larch is conspicuous by its absence, because it is abundant in areas not far removed from the bog. Here it exists in almost pure stands, where repeated fires have prevented other species from entering (Larsen, 1929). It played an important part in early postglacial forest succession in the vicinity of Spokane (Hansen, 1939).

While the climatic interpretation is tentative, it agrees essentially with that indicated by pollen analysis of bogs in the Puget Sound region and near Spokane. The general trend is also somewhat in agreement with that interpreted by some workers in the Middle West. Fuller (1935) believes that the mid-lake records indicate (1) a well marked period of increasing warmth; (2) a long period of maximum warmth; and (3) a poorly emphasized period of decreasing warmth. Von Post (1930) reaches

Table 1. Percentages of principal pollens.

Depth in meters	13	11 2/11 21	11	101/2	10	6/16	8 6	81/2	8 7	71/2	7 6	9 %9		51/2 5	5 41/2	/ <sub>2</sub> 4	31/2	3	21/2	c	11/2	1	1/2	t
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Tsuga heterophylla	•	:	:	•	:	:						-								13	4	9		11
Larix occidentalis	:	-	_	:	:	-														:	:	•		:
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\* Percentages are based upon the total significant pollens; the total significant pollens equal 100 per cent.

similar conclusions with respect to European postglacial climatic sequence. Sears (1932) basing his interpretation upon work in Ohio, Iowa, and Canada, concludes that there were several alternating periods of dryness and humidity, with a final warm and humid period. In northern Idaho the initial cool period gave way to one of warmth and desiccation and was succeeded by a period of increasing coolness and moisture, which, when once reached, has persisted to the present time. Forest succession in the Pacific Northwest is perhaps more dependent upon the relative tolerance of the species for shade than upon climatic change. This has probably been true during the latter part of postglacial time.

#### SUMMARY

Pollen analysis of a post-Wisconsin bog in Northern Idaho seems to indicate an initial postglacial forest of lodgepole and white pines, with an abundance of grass, marking a cool and medium dry period.

An abundance of Douglas fir and grass apparently reflects a second warmer and dryer period, which is also evidenced by analysis of a bog near Spokane, Washington.

A third period of increasing coolness and moisture is shown by the rapid increase in white pine to become dominant over lodgepole pine and the rapid decline of Douglas fir and grass.

This period was followed by one marking a trend toward climax development of hemlock, cedar, and white fir; and the decrease in the white pine subclimax. This trend apparently was interrupted, possibly by fire.

A fifth period of white pine increase is followed by a final period of slight decrease for the same species and another climax development trend. Climate probably has remained uniform since the maximum coolness and moisture of the third period was reached.

The interpreted climatic sequence in this region seems to correlate closely with that farther south near Spokane and to some extent with that in the Puget Sound region. The climatic interpretation is tentative until further work in the Pacific Northwest substantiates or refutes the foregoing conclusions.

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### NOMENCLATURE PROPOSALS FOR THE 1940 BOTANICAL CONGRESS 1

## F. R. Fosberg

The vast majority of botanists have little concern about the finer and more controversial points of nomenclature. They merely desire that the rules be understandable and definite in application and that they involve as little change in accepted nomenclature as possible. Ordinarily it matters little which way a question is decided, so long as the decision is definite and so worded that it is not necessary to have legal as well as botanical training to be able to interpret it.

Probably no set of rules will ever be drawn up with wording so perfect that no misinterpretations and ambiguities will appear later. These must be eliminated as they appear, in order that the rules may properly serve the purpose of clarifying con-

fusions in plant names.

It would seem better to point out certain inadequacies in the rules before the 1940 congress than to complain about them afterward. Therefore I am presenting here suggestions for discussion upon several articles which have, during the past four years, given trouble.

Nomina Provisoria.—In the first place, the term "nomen provisorium" (of Art. 37 ter. adopted at Amsterdam) should be defined in such a way that at least two botanists will be able to agree upon its meaning. I will leave it to someone who has convictions as to what it should mean to present a motion

on the question.

Proposal to amend Art. 30.—A fertile source of confusion has been the status of the subspecific or varietal epithets applied to the subdivision to which the type of a species belongs—the epithets typica, genuina, originaria, etc., of Recommendation XVIII. Under ordinary circumstances the question is not raised. Those who use these terms customarily treat them exactly as any other epithets of the same rank. Trouble comes only in those cases where an earlier varietal name has been applied to the form represented by the type of a species. Then the question arises as to whether this earlier varietal name should apply or whether it should be superseded by a var. typica or other such name.

Art. 30 says clearly enough that the earliest epithet of the proper rank should be used. No distinction is evident between ordinary epithets and those of the var. typica sort, but this is by no means agreed to by all botanists. At least two prominent American botanists have recently proposed the epithet var. typica when perfectly valid earlier varietal epithets appear in their synonymy (Munz, Bull. Torrey Bot. Club 64: 297, 1937; Fernald, Rhodora 39: 450, 1937). Editors, also, sometimes object to the use of an earlier varietal name to designate the "typical" form if it is based on a different type specimen than that of the species.

It has been explained to me that the expression var. typica is not a scientific epithet but merely a word to designate the typical subdivision of a species. It is maintained that this function is not performed by ordinary subdivisional epithets. It seems to me that any epithet is merely a word to designate a particular group of plants. Our rules are designed to govern the use of these words. There is nothing in the rules which says ordinary subdivisional epithets shall not designate the typical subdivision.

Of methods of dealing with these difficult cases, omitting consideration of those workers who prefer not to distinguish the typical form by any subdivisional epithet, there are at least three that have been used and one other suggested. First, there is that mentioned above, of giving epithets of the var. typica sort preference over all others and exempting them from the principle of priority. A second, which I have seen used lately (St. John and Hosaka, Occ. Pap. Bish. Mus. 14: 119, 1938), is to invoke Art. 60 case (1) and consider that the earlier varietal name, being unknowingly applied to the form containing the type of the species itself, was superfluous when published, therefore illegitimate. The third is to give var. typica exactly the same status as any other varietal name (St. John, Occ. Pap. Bish. Mus. 11, no. 13: 12-13, 1935; Fosberg, Madroño 4: 189-191, 1937), applying strict priority, but, of course, bearing in mind Recommendation XVIII, when creating new names. The fourth method, which has been suggested by Bolle (Notizbl. 13: 524, 1937), is to abolish all such terms as var. typica and simply repeat the specific epithet, with or without the prefix eu-, in all minor categories for the form containing the type.

Any one of these four solutions is perfectly workable. All that is required is a paragraph inserted in the rules designating one of them as the correct pro-

cedure.

The first method mentioned is directly contrary to Art. 30 of the rules as they now stand, as is also the fourth when an epithet of the proper rank is already in existence. In such cases they both violate

the principle of priority.

The second necessitates a very dubious interpretation of what makes a name superfluous when published. Art. 60 case (1) seems at best destined to cause as much confusion as it eliminates, without being applied to cases of this sort. Many botanists will argue that an epithet for the typical subdivision of a species is at any time superfluous.

The third of the above usages, though not innately superior to the others, is the only one that is thoroughly in accord with the rules as they now stand. It is the only one whose adoption will not amount to making an exception to the rules. The objection to having the species and its typical subdivision represented by different type specimens seems ground-

<sup>&</sup>lt;sup>1</sup> Received for publication January 6, 1939.

less; so long as the two types represent the same variety or form, this can cause no confusion, and as soon as they are shown to be different, a new name must be given one of them anyhow. This would naturally be given to the type of the species and would be of the var. typica sort. The proposition of Dr. Bolle (l.c.) for repeating the specific epithet would not be excluded in cases where no name of the proper rank already exists, as his procedure is permitted under the rules as they now stand (Art. 30 and Rec. XVIII).

As it will entail no change in the rules other than to make more definite their application, to provide a basis for discussion the following motion is presented to the section on nomenclature: Article 30 shall be amended by insertion of "Such subdivisional epithets as typica, originaria, genuina, etc., or subdivisional epithets which are repetitions of the specific name are to be treated, nomenclaturally, the same as any other epithets for groups below the

rank of species."

PROPOSAL TO AMEND ART. 36.—Names published in advertisements and nurserymen's catalogs have been the source of much uncertainty, both because of the poor descriptions that often accompany them and because it is sometimes argued that they cannot be considered validly published. An example is the publication of four "new species" of Eranthemum by Wm. Bull in an advertisement in Gardener's Chronicle n. s. 3: 619, 1875. According to a strict interpretation of the rules these would have to be considered as effectively published, but there has been much ambiguity in the use of the names there published. The best way of eliminating the confusion caused by such names seems to be to consider all names published in advertisements ineffectively published. Obviously, if they had been intended as scientific publications, the medium of advertisements would not have been used.

This may be accomplished by adopting the motion here proposed: To amend Article 36 by adding "Seedsmen's and nurserymen's catalogs or other obvious commercial advertisements, whether in a periodical or privately distributed, are not to be considered effective means of publication, even though the names used in them are accompanied by descrip-

tions."

Proposal to amend Art. 60.—When a name or epithet previously invalidly published is validated by a subsequent author but ascribed to the author who published it first, the question sometimes comes up as to whether the application of the name is to be determined by the intention of the original author, if determinable, or by the sense in which the name is used by the author who validates it. Thus, if Eranthemum atropurpureum Bull, in Gard. Chron. 18. 3: 619, 1875, is considered invalid, either as a nomen subnudum or because published in an advertisement, Hooker's use of the name in Bot. Mag. t. 7839, 1902, with an excellent description, must be regarded as a validation of it, as Hooker refers to Bull as the author of the name. Radlkofer in Sitzb.

Math.-Phys. Cl. Acad. Wiss. Muench. 13: 286, 1884, transferred the species to *Pseuderanthemum*. Bailey in Gentes Herb. 1: 130, 1923, considered that Hooker had described a different plant from that which Bull had in mind (on the basis of a specimen deposited at Kew by Bull many years after the publication of the name and by no means necessarily the same plant as that described in 1875). He therefore retained the name *Pseuderanthemum atropurpureum* for Bull's plant and renamed Hooker's plant *Ps. kewense*.

Radlkofer's combination is, of course, invalid, as the only reference is to Bull's nomen subnudum. Bailey (l.c.) remade the combination, specifically stating that it was for Bull's plant, not Hooker's. However, in view of Article B54, as adopted at the Amsterdam congress (Proc. Int. Bot. Congr. Amsterdam 1: 354, 1936), it seems that Bailey's combination should be applied to Hooker's plant. Ps. kewense Bailey is, in any event, illegitimate; it was superfluous when published. Bailey should have used Hooker's name for it and renamed Bull's plant. Bull's plant is without a name.

To avoid such situations in the future, the following motion is presented: To Article 60 add: "The application of a name is to be determined by the sense in which it is used in its first valid publication. Previous invalid publications do not affect it excepting only when they are useful in doubtful cases as a help in determining the application in the

earliest valid publication."

Two generic names proposed for conserva-TION.—The names Artocarpus Forst., Char. Gen. 101, 1776, and Inocarpus Forst., Char. Gen. 66, 1776, are hereby proposed to the Special Committee for Phanerogamae and Pteridophyta for inclusion in the list of Nomina Generica Conservanda. Both are antedated by names legitimately published three years earlier by Parkinson in "A Journal of a Voyage to the South Seas in His Majesty's Ship, The Endeavor," 1773. The earlier name for Artocarpus Forst. is Sitodium Parkinson, l.c. p. 45, and that for Inocarpus Forst. is Aniotum Parkinson, l.c. p. 39. Both Parkinson names are published with combined generico-specific descriptions (see Art. 43), with binomials and in a work in which binomials are consistently used. To any one familiar with the Polynesian flora there can be no ambiguity as to the application of the names, and the descriptions are as complete as those in many accepted works of the time. Taken with the supplementary data available (see Merrill, in Jour. Arnold Arboretum 19: 301-2, 1938), they are positively identifiable.

Following is a concise statement of the cases for conservation of these two genera, for the use of the committee. Though Article 21, note 1, of the rules requires a statement of the cases both for and against conservation of proposed names, there seems to be no case whatever against conservation of these names, excepting perhaps the small size of the genus

Inocarpus.

Artocarpus versus Sitodium.—Artocarpus (Moraceae) is a genus of about 40 species of tropical Asia to Polynesia and cultivated in all tropical regions. Its conservation over Sitodium seems warranted by the facts stated below.

The genus includes several well known economic species, such as the common bread-fruit and the jak-fruit, and the name is in common use by horticulturists, travelers, and ethnologists, as well as by botanists. A change would cause great confusion to these people.

The name Artocarpus has been in continuous use since 1776, while Sitodium has been used only by Gaertner in one publication since it was first published.

Index Londinensis lists 192 plates under the name Artocarpus against 1 under Sitodium.

Important general works such as DC. Prodromus 15 (1): 264, 1864; Benth. & Hook., Gen. Pl. III, 1: 376, 1880; Engl. & Pr., Die Nat. Pflanzenf. Ed. I, III, 1: 82, 1889; such important regional floras as Trimen's Handb. Fl. Ceyl. 4: 98, 1898; Miquel, Fl. Ned. Ind. 1 (2): 284, 1859; Merrill, Enum. Phil. Fl. Pl. 2: 40, 1923; Seemann, Fl. Vit. 255, 1873; Rock, Ind. Tr. Haw. Is. 115, 1913; such important horticultural works as Bailey, Stand. Cycl. Hort. 1: 401, 1928; and such important morphological works as Janssonius, Mikrogr. 6: 241, 1936, all adopt Artocarpus.

Inocarpus versus Aniotum.—Inocarpus (Leguminosae) is a genus of perhaps three species, of tropical Asia to Polynesia and Guiana (?) and widely planted as a food plant in the Pacific islands and Asia. Its conservation over Aniotum seems warranted by the facts stated below.

The principal species of the genus is an important economic plant in tropical regions, as well as a morphological curiosity, anomalous in its family, and the name is in common use by horticulturists and ethnologists, as well as by botanists. A change would cause great confusion to these people.

The name *Inocarpus* has been in continuous use since 1776, while *Aniotum* has been used only by Gaertner in one publication since it was first published.

Index Londinensis lists 10 plates under the name Inocarpus and none under Aniotum.

Important general works such as DC., Prodromus 17: 284, 1873; Benth. & Hook., Gen. Pl. 1: 552, 1867; Engl. & Pr., Die Nat. Pflanzenf. Ed. I, III, 3: 348, 1894; such important regional floras as Miquel, Fl. Ned. Ind. 1 (1): 887, 1855; Merrill, Enum. Phil. Fl. Pl. 2: 302, 1923; Brown, B. P. Bish. Mus. Bull. 130: 118, 1935; and such important morphological works as Janssonius, Mikrogr. 3: 82, 1918, all adopt *Inocarpus*.

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## RESPONSE OF LETTUCE SEEDLINGS TO 7600A RADIATION 1

Lewis H. Flint and Charles F. Moreland

In studying the effect of spectral light upon the germination of light-sensitive lettuce<sup>2</sup> seed, Flint and McAlister (1935) found that a band of radiation at about 7600A inhibited the germination. Numerous researches had previously given results in which unfavorable effects had been attributed to general near infra-red radiation, but in these experiments the range of the effective radiation was fairly well defined.

In studying the phoeolic movements of Mimosa pudica in relation to intensity and wave length, Burkholder and Pratt (1934) also found near infrared radiation of about the same critical wave length maximally effective. The two sets of data were graphically brought together for comparison by Burkholder in his survey of light in relation to plant growth (1936). The order of agreement indicated by this comparison appeared sufficient to suggest that the near infra-red radiation at 7600A might have a more general influence on plants. The experiments here reported were carried out in line with this suggestion.

Метнорз.—In these experiments light-sensitive lettuce seeds were placed on moist filter paper in

<sup>1</sup> Received for publication January 16, 1939.

<sup>2</sup> Lactuca sativa L.

Petri plates and exposed to red light at 20°C. for 48 hours. At this time uniformly-germinated seedlings were selected and arranged on moist cotton or filter paper in small moist chambers for exposure to 7600A radiation. In the first series of trials the 7600A radiation was introduced at a horizontal incidence in a dark chamber, the seedlings outside the beam thus being in total darkness and constituting the control or check plants. In the second series of trials the 7600A radiation was introduced into the dark chamber at a vertical incidence, the plants outside the beam again constituting the checks. Several variations in the manner of exposure were included with a view to reducing chance as a factor in the indicated response. A trial with radiation at 6500A and another with radiation at 8500A were included to indicate something of the specificity of the response to 7600A radiation.

In these experiments a Mazda 1000-lumen street lamp was used as the light source for the continuous radiation. Suitable combinations of intensity and monochromacity of radiation were obtained with a reflecting monochromator. The intensity was measured with a vacuum thermocouple and galvanometer. There was no control of temperature within the exposure chamber, and this would sometimes vary sev-

Table 1. Results obtained with 7600A radiation treatments at an intensity of approximately 600 ergs per sec. per cm<sup>2</sup>. Wave length range 7065A to 8135A. Lettuce seedlings.

Nu	mber of s	eedlings use	d — Average he	ight in mm	
Nature of exposure (vertical incidence)	Control area	Treated area	Control	Treated	T-C
Treated plants in center of plate surrounded by control plants	65	50	27.4	21.3	.78
Treated plants in center of plate surrounded by control plants	120	35	21.65	13.85	.64
Treated plants in center of plate; con-	50	10	$     \begin{bmatrix}       29.5 \\       30.9 \\       28.2     \end{bmatrix}   $	20.7	.71
trol plants in five groups	<b>5</b> 0	10	29.9 27.2	20.1	./1

eral degrees during the course of an experiment. However, no differences in temperature were detectable in the treated and check areas occupied by the seedlings.

RESULTS.—In the first series of trials with lettuce seedlings subjected to 7600A radiation at horizontal incidence, no bending was obtained. Follow-up trials with maize coleoptiles also gave no bending. As a further check, the lettuce seedlings were exposed to radiation at 4800A, and pronounced bending took place rapidly. It was thereupon concluded that there was no phototropic response to 7600A radiation.

In the second series of trials the lettuce seedlings were subjected to 7600A radiation at vertical incidence. The first experiments of this series gave the results brought together in table 1.

It was considered that several objections might be made to the arrangements indicated in table 1. The seedlings exposed to the 7600A radiation were in the center of a disc and might therefore be exposed to inhibiting substances exuded from the surrounding plants. To overcome this objection an arrangement was chosen which permitted a random selection of the group of seedlings exposed to the radiation. The seedlings were situated about seven feet from the ordinary S-type Mazda burning at 12 volts, and, as previously stated, no temperature differences between the exposed area and that adjacent to it could be detected with a thermometer. Yet to make certain that the effect of the 7600A radiation was not simply due to a drying out of the area, some experiments were included in which water was left standing above the moist filter papers in the Petri

Table 2. Results obtained with radiation at 7600A, 6500A, and 8500, respectively. Lettuce seedlings.

	Critical wave length	Number o	f seedlings	A wono no h	.:	
Nature of exposure (vertical incidence)	Wave length range Order of intensity	Control area	Treated area	Control	Treated	T-C
Plants arranged in six groups of ten each; random selec- tion of one group for treat- ment		5 × 10	10	$ \begin{cases} 11.0 \\ 11.1 \\ 11.3 \\ 11.5 \\ 11.0 \end{cases} $	9.1	.815
Plants arranged in six groups of ten each; random selec- tion of one group for treat- ment	$ \begin{cases} 7600 \text{A} \\ (7422 \text{A} - 7778 \text{A}) \\ 200 \text{ ergs/sec/cm}^2 \end{cases} $	5  imes 10	10	$ \begin{cases} 20.6 \\ 19.3 \\ 18.7 \\ 18.2 \\ 19.2 \end{cases} $	16.3	.85
Plants arranged in six groups of ten each; random selec- tion of one group for treat- ment	$   \begin{cases}     6500A \\     (6282A-6718A) \\     300 \text{ ergs/sec/cm}^2   \end{cases} $	5 × 10	10	\begin{pmatrix} 18.4 \\ 19.3 \\ 18.6 \\ 18.8 \\ 18.0 \end{pmatrix}	18.8	1.009
Plants arranged in six groups of ten each; random selec- tion of one group for treat- ment	$ \begin{cases} 8500A \\ (8253A-8747A) \\ 256 \text{ ergs/sec/cm}^2 \end{cases} $	5 × 10	10	21.7 20.9 22.2 21.6 21.4	21.3	0.987

dishes. The same stunting effect was obtained under these conditions. Another objection to the experiments as given in table 1 is that the stunting effect may be only a part of a more general influence extending into the infra-red. To test this possibility, some experiments were carried out with radiation at 8500A. No stunting effect whatever was obtained with this radiation.

Yet another objection to the experiments of table 1 is that the stunting effect may be associated with assimilation. The assimilation curve of Hoover (1937) indicated that the long wave length limit of radiation promulgating assimilation was about 7400A. This point was well within the range involved in the treatments at 7600A. To satisfy this objection, the slit-widths of the monochromator were so reduced as to produce an emergent beam devoid of any radiation effecting assimilation. When trials with this beam still gave stunting effects, an additional trial was run with increased energy at 6500A—the maximum of Hoover's assimilation curve. The results of these supplementary experiments have been brought together in table 2.

It will be noted from the results given in table 2 that while the stunting effect was less marked at the reduced energy, there was no stunting effect either at 6500A, the point of maximum assimilation in Hoover's curve, or at 8500A, a point further into the infra-red range. There remains the possibility that the stunting effect of 7600A radiation might be nullified when coupled with 6500A radiation; but since the latter did not influence elongation when compared with darkness, although greening took place, this possibility appears rather remote.

The general characteristics of the radiations used in these experiments, together with a portion of Hoover's curve of assimilation, are indicated in the accompanying graph (fig. 1).

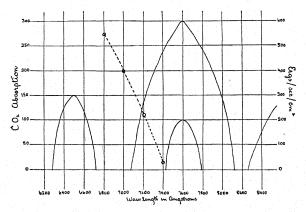


Fig. 1. Characteristics of radiations used in experiments with lettuce seedlings. Solid line: radiations, plotted against scale at right; broken line: portion of assimilation curve of Hoover, plotted against scale at left.

The stunting effect of the 7600A radiation as obtained in these experiments is indicated in the accompanying photograph (fig. 2).

Discussion.—The 7600A radiation, which was found to inhibit the germination of light-sensitive lettuce seed, also appears to stunt the development of the seedlings. This stunting effect does not appear to be associated with assimilation, nor with temperature. The extent to which this radiation may

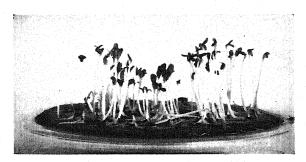


Fig. 2. Stunting effect of 7600A radiation on lettuce seedlings. Plants in center exposed to radiation.

have a similar effect upon other plant material is not known.

In accordance with a long-established principle the material responding to 7600A radiation might be expected to contain pigment absorbing this radiation. Up to the present time no satisfactory data indicating such an absorption have been obtained. It may be noted that in figure 2 the exposed seedlings have a somewhat darker appearance than the controls. This darker appearance was less noticeable to the eye. There has never been obtained any greening in plants exposed to the 7600A radiation.

Conclusions.—Radiation at 7600A stunted the the growth of lettuce seedlings. The effect was not associated with assimilation nor with temperature. Radiation at 6500A and at 8500A had no stunting effect.

Radiation at 7600A incited no phototropic response in lettuce seedlings.

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## INFLUENCE OF LIGHT ON THE LENGTH OF THE CONIDIA IN CERTAIN SPECIES OF FUSARIUM <sup>1</sup>

L. L. Harter

A CONSIDERABLE amount of literature has accumulated on the influence of light and radiation on fungi, which for the most part deals with a variety of physiological processes but in few, if in any case, with the direct influence of light and dark periods on the size of the spores, as shown by actual measurements. Appel and Wollenweber (1910) found that when Fusarium cultures were grown in the dark, the conidia were often abnormal in form and variously septated. Ramsey and Bailey (1930) found that a strain of Fusarium coeruleum (Lib.) Sacc. that had never produced conidia was induced to do so when a culture was exposed to ultra-violet radiation and that under the same treatment a culture of Fusarium argillaceum (Fr.) Sacc. produced only chlamydospores. Stevens (1928, 1931), working with a different group of fungi, showed that radiation from a quartz-mercury vapor lamp induced the development of perithecia in cultures of Glomorella cingulata (Atk.) S. and S., Colletotrichum lagenarium (Pass.) Ell. and a species of Coniothyrium. Smith (1935) also found that short exposures of cultures to ultra-violet radiation caused a marked stimulation in spore production. Ramsey and Bailey (1930) as well as Smith demonstrated that the increase in spore production did not seem to be correlated with any inhibition of the vegetative growth. Of interest also are the results of Brown (1925) to the effect that Monilia fructigena (Pers.) Schr. in general showed a tendency to sporulate in the light, and those of Coons and Levin (1920) that pycnidia formation was stimulated in different genera of the Sphaeropsidales by the action of two 100-watt nitrogen filled incandescent lamps. As an explanation of their results, Coons and Levin suggested that the effect of the light on the mycelium of Plenodomus fuscomaculans (Sacc.) Coons served as an oxidizing force, unlocking the reserves of cellular food, thus furnishing the energy for the fruiting processes.

It is evident from the literature already cited that previous studies on the effect of light on fungi have dealt mostly with its influence on color production, vegetative growth, and spore population rather than

on the actual size of the conidia.

The problem.—The data to be reported in this paper are the results of the writer's efforts to explain in part at least why he has been unable in many cases to identify correctly certain species of Fusarium with which he worked or which came under his observation. The investigations of Wollenweber (1931) and Wollenweber and Reinking (1935) give complete descriptions of the different species of Fusarium with numerous measurements of the length and width of the macroconidia and microconidia, size of the chlamydospores, as well as color reactions on different culture media and other

<sup>1</sup> Received for publication January 30, 1939.

characteristics of the organisms that might enable the student to separate one species from a closely related one. In spite of these details, it is often difficult, if not impossible, by any means of manipulation of the culture to obtain a morphological agreement with the description of a given species. In fact a spore or clonal transfer from a type culture does not always give a culture whose spores or other characteristics agree with the original. Differences in spore size as great as that between two distinct species sometimes result from such a transfer. Other difficulties are frequently encountered in pathological or mycological work where species of Fusarium are involved. Different isolates from the same host when collected either from the same or a different locality do not always agree morphologically with the original description of the species. These difficulties are further increased when species, varieties, or forms are separated on the basis of differences of a few microns in the average length of the conidia. The difficulties experienced by the writer have also been experienced by many other investigators who have attempted to identify species by using the recognized standards of morphological relationship. Many of the difficulties of the classification of species of Fusarium as formulated by a Fusarium conference in Madison, Wisconsin, in 1924 have been concisely stated by Nelson, Coons, and Cochran (1937), who feel that morphological characters alone are not sufficient to enable anyone but a specialist on this genus to segregate species.

The lack of any uniform methods of handling cultures has doubtless contributed something to the confusion. Little has been attempted in the way of studying the effects of various agencies such as light, temperature, etc., on the size and shape of the conidia, although it has been recognized that they influence the reproductive behavior of the organism.

The writer believes it can be shown that photoperiodism influences the length of the conidia of certain species of Fusarium independently of other agencies and that the mean length of spores taken from different culture tubes derived from the same clonal line differs greatly when the organisms are reared under otherwise identical conditions. It is believed that these investigations will explain, in part at least, why so much difficulty, confusion, and incorrect identification have been experienced in this important group of fungi.

Species of Fusarium investigates.—Three species of Fusarium have been employed—namely, F. coeruleum, F. Martii var. pisi Jones, and F. bulbigenum var. batatas Wollenw. These three species were selected principally because they had continued to produce pionnotes and sporodochia for several years on several different culture media—a prerequi-

site in work of this kind. Fusarium bulbigenum var. batatas, however, did not maintain its ability to form sporodochia to quite the same degree as the other two species, especially on certain culture media. For that reason the data with respect to this species are not so complete as for F. coeruleum and F. martii var. pisi.

Fusarium coeruleum (I.K. 6721)<sup>2</sup> was isolated from the roots of peas in Colorado by W. J. Zaumeyer in 1932. It forms a copious, dark indigo blue pionnotes on cooked potato, stringbeans, 2 per cent potato-dextrose agar, and most of the culture media in common use, and can be maintained in such a state of growth without difficulty. It is the cause of a root rot of peas (Harter, 1938). Because of the constancy in its growth behavior, it has been admirably suited for the study of the influence of light and dark on the length of the spore.

Fusarium martii var. pisi (I.K. 6865a), the cause of pea root rot, is a descendant from the original culture made by F. R. Jones, who first studied the disease and described the organism. It produced green pionnotes or sporodochia on cooked potatoes, potato-dextrose agar, bean pods, and certain other media. It does not produce the copious pionnotes produced by F. coeruleum and is somewhat more difficult to maintain in "high culture." By frequent transfers to suitable media, it has been maintained in a condition satisfactory for the problem under investigation.

Fusarium bulbigenum var. batatas (I.K. 7313c), a representative of the section Elegans, is a vascular parasite and the cause of the wilt or stem rot of sweet potato (Harter and Field, 1914). It forms a copious pionnotes on cornmeal agar, 2 per cent potato-dextrose agar, and other media when first isolated, but later the spore masses were reduced to well developed sporodochia. This species was more difficult to maintain in high culture than either of the two others, or other species of the section Martiella.

EXPERIMENTAL PROCEDURE.—Preparatory to starting the investigations, each organism was "purelined" by picking a single conidium from an agarplate colony and transferring it to another agar plate or to some suitable culture medium in a test tube. These lines were used as a source of all subsequent transfers. Stock cultures were maintained on a medium found to be the best for the production of pionnotes and were grown in the light, generally on the ledge of a north window, in a room where the temperature varied from about 15°C. to 24°C. during the winter months.

All spore measurements were made from cultures grown in test tubes. All agars were slanted and the inoculum smeared the length of the slant. When cooked potatoes, stringbeans, or similar media were inoculated, the inoculum was spread as much as possible over the surface in order to obtain as uniform a distribution of the spores as possible. In all trials

<sup>2</sup> Isolation Key Number 6721 carried in the files of L. L. Harter. care was exercised to select test tubes of uniform diameter.

The culture media were inoculated as follows: about the amount of spore material that would fill a 1 mm. platinum loop was transferred from a stock culture tube to 2 cc. of sterile water in a test tube. The spore suspension was then shaken vigorously to insure a uniform dispersion of the conidia in the water. From this spore suspension, a single transfer was made to the tubes to be used for exposure to the different light values by the use of a number 24 B & S platinum wire.

Inasmuch as these experiments have to do with the influence of various durations of light and dark on the length of the conidia, the methods of preparation and manner of exposure will be described in considerable detail. Each tube to be kept in the dark for the entire length of the experiment or for selected intervals was wrapped with six thicknesses of black paper such as is used to wrap unexposed photographic plates or films. The paper extended from the upper end of the test tube to about two inches below it. The portion of the paper that extended below the tube was folded over to shut off any light from the bottom. The paper did not extend above the tube or over the cotton plug so that there was the same opportunity for the exchange of air as in the unwrapped tubes. A small amount of light may have entered the tube through the cotton plug, but it is believed to be so small as to be negligible. When the tubes were exposed to solar irradiation, the wrapper was removed for the desired length of time and replaced at the end of the light exposure period.

The investigations were divided into two parts: (1) those in which solar irradiation was used as a source of light; (2) those in which artificial light was employed. In the latter case the cultures were kept in incubators with light excluded, except as it was supplied artificially in a known intensity and for a selected duration of time.

The incubators were well insulated boxes  $3 \times 2 \times 2.5$  feet in size and black inside. They were located inside an insulated, practically light-proof refrigeration room maintained at a temperature of  $9-10^{\circ}$ C. The refrigeration room and the incubators were thermostatically controlled. Fans were used in the refrigeration room and in the incubators to keep the air in constant motion. The open wire crates used to hold the cultures were placed on a wire shelf about 1 foot above the incubator floor, thereby providing for free circulation of the air around the tubes.

Two-hundred-watt Mazda lamps hung outside the boxes 26 inches from the cultures were the only source of illumination. The duration of the illumination was regulated to suit the experiments. The length of the light and dark periods will be shown as the results of the various experiments are presented.

In those experiments conducted in the laboratory the cultures exposed to the light of a north window were subjected to temperatures varying from about 15° to 24°C. A temperature of 25°C. was employed for most of the experiments where the cultures were exposed to artificial illumination, since a series of experiments with the different species conducted at temperatures of 14°, 18°, 22°, 25°, and 27.5°C. had shown that those grown at 25°C. were, everything considered, equal to if not superior to those grown at the other temperatures. At 27.5°C. the cultures and the conidia were not quite normal, showing that the temperature was probably a little too high. Those grown at 14°, 18°, and 22°C. were very similar in general appearance to those grown at 25°C., but the growth was correspondingly slower at the lower temperatures. Everything else being equal, the temperature giving the most rapid normal development was preferred, and that temperature was 25°C. Pionnotes developed 1 to 2 days later at 22°C. than at 25°C., 4 or 5 days later at 18°C., and about 8 days later at 14°C. The attainment of the reproductive stage in the growth cycle of the different species in the shortest time possible was a matter of considerable importance.

Several tubes of media were inoculated at one time with each organism for each type and length of exposure. Growth was evident in the three species in two days. Pionnotes were forming in cultures of Fusarium coeruleum in 4 days, in F. martii pisi in about 6 days, and in F. bulbigenum var. batatas several days later. In spite of the fact that the spores of F. coeruleum appeared to be fully developed and in every way normal in 10 days, no measurements were made from cultures less than 21 days old. A

correspondingly longer time was allowed for the two slower-growing species.

After a careful consideration of several methods. it was decided to measure the length of 100 3-septate spores chosen at random from each of several culture tubes. A spore suspension was made in water on a glass slide and then diluted to a point where only two or three conidia could be observed in one field of the microscope at the combination of oculars and objectives used in making the measurements. When the desired spore dilution was reached, a cover glass was put on the mount and sealed around the edges with paraffin to hold the spores in place and prevent the water from evaporating. With such a preparation, two or three hours were available for making measurements before the conidia began to germinate. It is believed that if a thin distribution of spores is made on a slide, a reliable sample can be obtained by measuring each 3-septate spore as it comes into the field of the microscope. The method was adopted of starting at one side of the preparation, moving the slide in only one direction and measuring every 3-septate spore that came entirely into view.

EXPERIMENTAL RESULTS.—The spore measurements are recorded in microns. It was believed that a better impression of spore size could be obtained by measuring 100 spores from each culture and recording the number that fell into each micron class, rather than by recording only the maximum, minimum, and average, as most workers with Fusarium have been accustomed to do. The data shown in the several tables are the results of measurements made

Table 1. Frequency distribution of the length measurements of 3-septate conidia of Fusarium bulbigenum var. batatas grown at room temperature with solar irradiation.

				Frequen	cy distr	ibution			
	Culture	s on cor agar	nmeal	Cultures be	on cook ean pod		Culture potato-	s on 2 p -dextros	
Length in microns	a	ь	e	a	ь	e	a	b	c
22.5			5	•					
25.0			19			24		5	1
27.5			43	2	2	53	4	16	18
30.0	4	3	108	8	14	76	31	63	67
32.5	24	28	126	35	54	111	90	103	55
35.0	76	56	76	83	88	97	120	107	43
37.5	97	89	21	103	92	34	98	71	15
40.0	97	77	2	48	39	5	44	25	1
42.5	74	36		18	11		10	10	
45.0	23	. 8	• •	3		K .	3		
47.5	5	2		• •					
50.0		1	••						
Number of conidia	400	300	400	300	300	400	400	400	200
Means <sup>d</sup>	38.7	38.0	31.6	36.7	36.0	32.0	35.4	34.1	32.1
SE <sub>m</sub>	±.18	$\pm .19$	$\pm .16$	±.18	±.17	±.17	±.16	$\pm .18$	$\pm .20$
C.V.*	9.3	8.6	9.8	8.3	8.2	10.6	9.1	10.5	9.0

<sup>\*</sup>Unwrapped; exposed to solar irradiation. b Exposed to daylight 9 to 12 a.m. daily. Wrapped continuously in black paper. d Expressed in microns. Expressed in percentage.

Table 2. Frequency distribution of the length measurements of 3-septate conidia of Fusarium coeruleum grown at room temperature with solar irradiation.

•	Frequency distribution													
	Cultures on cornmeal agar			Cultures on cooked green bean pods			Cultures on 2 per cent potato-dextrose agar			Cultures on cooked potato				
Length in microns	a	b	c	a	b	c	а	b	c	А	b	c		
22.5	1	•••	5			3	•••		2	••	A 44			
25.0	3		11		1	36			17		2	9		
27.5	2	12	86	1	5	84			55	1	9	35		
30.0	14	42	99	1	19	99	1	1	99	15	49	84		
32.5	19	69	63	2	38	55	3	6	77	32	102	99		
35.0	41	88	29	9	51	20	16	36	34	75	128	60		
37.5	72	56	6	41	46	2	37	52	11	123	72	13		
40.0	76	24	1	76	30	1	71	49	5	93	30			
42.5	4.2	8		63	7		81	33		45	7 .			
45.0	21	1		61	3		51	14		13	1			
47.5	6			430			21	8		3				
50.0	4			14			19	1						
52.5				1			• •				• •	• •		
55.0				1			• •							
Number of conidia	300	300	300	300	200	300	300	200	300	400	400	300		
Means <sup>d</sup>	38.5	34.5	30.2	42.2	35.5	29.5	42.0	39.3	30.9	37.8	34.5	31.7		
SE <sub>m</sub>	±.25	$\pm .20$	$\pm .17$	±.23	$\pm .26$	$\pm .17$	±.22	$\pm .25$	$\pm .16$	$\pm .17$	$\pm .16$	$\pm .16$		
C.V.e	11.4	10.1	9.9	9.4	10.4	9.9	9.3	8.8	8.9	9.2	9.4	8.7		

<sup>&</sup>lt;sup>a</sup> Unwrapped; exposed to solar irradiation. <sup>b</sup> Exposed to daylight from 9 to 12 a.m. daily. <sup>c</sup> Wrapped continuously in black paper. <sup>d</sup> Expressed in microns. <sup>e</sup> Expressed in percentage.

from at least two and mostly from five, six, and seven tubes.

Solar irradiation.—The cultures exposed to solar irradiation at room temperature were placed on the ledge of a north window and consequently were subjected to the changes of temperature and light intensity that would naturally occur from time to time. Black paper was used to wrap the culture tubes from which the light was to be excluded for a part or all of the growth period. While the light intensity and temperature were not controlled except in a general way, the cultures were otherwise identical in every respect except that one set was exposed to light and the other wrapped in black paper. Notwithstanding the fact that conditions surrounding the cultures were not accurately controlled, the effect of the different periods of exposure is so striking as to leave little doubt that light exercises a profound influence on the septation and size of the conidia (tables 1 to 3).

The data shown in tables 1 to 3 are the results of exposure of three different species of Fusarium to daylight for different durations of time. Three series were run at one time. One was exposed constantly to all the available daylight, one from 9:00 a.m. to 12:00 noon daily, and one was wrapped in black paper to exclude the light. An examination of these figures shows a consistent trend so far as the length of 3-septate spores is concerned, irrespective of the medium on which the organisms were grown. The greatest mean length of conidia occurred in cultures exposed to the maximum amount of avail-

able daylight under the conditions of these experiments. The conidia next longest in mean length were obtained from cultures exposed to daylight daily from 9:00 a.m. to noon, and the shortest were in the culture tubes wrapped with black paper. The mean length of 400 conidia of Fusarium bulbigenum var. batatas grown on cornmeal agar in daylight and in the dark was 38.7 and 31.6 microns, respectively (table 1). Less significant differences were obtained when identical cultures were grown on green bean pods and on 2 per cent potato-dextrose agar. The difference between the mean length of the conidia of cultures grown in maximum daylight and of those exposed to daylight for 3 hours daily is not so striking as it is between daylight-grown cultures and cultures grown in the dark, but nevertheless is impor-

Equal or even greater differences in the mean length of the conidia are shown in the case of Fusarium coeruleum (table 2) grown on 2 per cent potato-dextrose agar, the length of the spores grown in daylight and in the dark being 42.1 and 30.8 microns, respectively. Variations almost as great occur when this organism is grown on cornmeal agar and cooked potatoes. Fusarium martii var. pisi (table 3), regardless of the culture media used, also showed a decrease in the length of the conidia more or less inversely proportional to the amount of daylight to which the cultures were exposed.

ARTIFICIAL IRRADIATION.—The data shown in tables 4 and 5 were obtained from cultures grown in an incubator with a temperature of 25°C. In all

Table 3. Frequency distribution of the length measurements of 3-septate conidia of Fusarium martii var. pisi grown at room temperature, in the dark and with solar irradiation.

Frequency distribution												
	Cultures on cornmeal agar			Cultures on cooked green bean pods			Cultures on 2 per cent potato-dextrose agar			Cultures on cooked potato		
Length in microns	a	b	c	a	b	c	a	b	c	a	b	c
22.5			1			4		• •		••		13
25.0	.:		6			47			7		16	60
27.5	1	6	19		1	119		3	36	22	68	157
30.0	16	16	72	11	25	134		22	88	76	157	155
32.5	29	36	105	20	51	73	12	45	153	148	161	69
35.0	36	53	91	38	64	17	30	86	153	124	113	35
37.5	65	61	63	98	85	5	48	156	93	90	60	10
40.0	94	58	30	110	50	1	82	133	49	28	19	1
42.5	100	41	11	117	18		123	109	16	9	6	
45.0	106	20	2	77	5		109	35	3	2	• • • •	
47.5	38	7		21	1		65	10	2	1		
50.0	13	2		8			25	1		• •		
52.5	2						6					
Number of conidia	500	300	400	500	300	400	500	600	600	500	600	500
Means <sup>d</sup>	41.0	37.8	33.9	40.4	36.3	29.4	42.6	38.5	34.1	34.1	32.4	29.3
SE <sub>m</sub>	±.21	$\pm .26$	$\pm .19$	±.18	$\pm .21$	$\pm .14$	±.19	$\pm .15$	±.15	$\pm .15$	$\pm .14$	$\pm .14$
C.V.e	11.5	12.1	11.2	10.1	10.0	9.5	9.9	9.8	11.2	10.0	10.9	10.9

<sup>&</sup>lt;sup>a</sup> Unwrapped; exposed to solar radiation. <sup>b</sup> Exposed to daylight from 9 to 12 a.m. daily. <sup>c</sup> Wrapped continuously in black paper. <sup>d</sup> Expressed in microns. <sup>e</sup> Expressed in percentage.

Table 4. Frequency distribution of the length measurements of 3-septate conidia of 3 species of Fusarium grown at 25°C. temperature in the dark and with continuous artificial irradiation.

	Frequency distribution											
		Fus	sarium e	coeruleu	m		Fusa	rium ma	. pisi	Fusarium bulbigenum var. batatas		
	Cultures on cooked potato		Cultures on cooked bean pods				n 2 per cent xtrose agar		Cultures on cooked potato		Cultures on corn- meal agar	
Length in microns	а	b	a	b	a	b	a	ъ	a	b	a	b
22.5		25		44		1			•••	5		13
25.0		106		123		47		11		34	1	45
27.5	4	167	1	117		156	1	74	5	81	25	83
30.0	28	87	17	80	6	145	6	114	23	106	82	172
32.5	90	15	44	34	30	46	11	110	46	57	177	227
35.0	95		66	2	52	5	32	58	47	14	204	124
37.5	112		88		89		55	21	72	3	158	33
40.0	45		90		98		77	8	76		43	3
42.5	19		53		86		90	4	28		1	
45.0	5		32		36		83		3			
47.5	1		8		3		33		•			
50.0	1		1	• •			12					
Number of conidia	400	400	400	400	400	400	400	400	300	300	691	700
Means <sup>e</sup>	35.8	27.3	38.2	27.1	39.1	28.8	41.4	31.5	36.8	29.4	34.4	31.3
$SE_m\ \dots\dots$	±.18	±.11	±.21	±.14	±.19	±.11	±.21	±.17	±.20	±.16	±.12	±.13
C.V.4	10.0	8.4	10.9	10.7	9.6	8.3	10.4	10.8	9.4	9.5	9.0	10.8

<sup>\*</sup> Artificial irradiation 24 hours daily. b Dark 24 hours daily. Expressed in microns. Expressed in percentage.

cases the light was derived from a 200-watt filament Mazda lamp 26 inches distant from the culture tube.

In general the results in an incubator where both the temperature and illumination were controlled were almost identical with those obtained in a laboratory room in which there was no accurate control of either temperature or light intensity. A consideration of some of these data suggested that a similar stimulation of fructification and increase in the length of the spore might be induced by irradiating the culture for only a part of the time following inoculation. With this thought in mind, experiments (table 4) were conducted in which some of the cultures were irradiated for 24-hour periods daily. while others were kept continuously in the dark. In another series (table 5) the cultures were irradiated two and four days immediately following inoculation, after which the light was excluded (dark) for the remainder of the incubation period. In other experiments (table 5) the cultures were kept in the dark for a definite period of time immediately following inoculation, after which they were exposed to continuous light during the last few days of the growth period. Concomitantly with the above experiments, another set of cultures (table 5) was subjected from the start to alternate light and dark periods of 12 hours each in comparison with a similar set of cultures kept continuously in the dark from the beginning.

The effect of artificial illumination in increasing the mean length of the conidia of three species of Fusarium has been fully established by the results shown in table 4. In the solar irradiation experiments (tables 1 to 3) it was found that the conidia of cultures exposed for 3 hours daily had a longer mean length than those of cultures from which the light had been entirely excluded, but these data gave no indication with respect to the period in the life cycle of the fungus when illumination was most effective. When cultures of Fusarium coeruleum that were kept in the dark for the first two days and then artificially irradiated for the remainder of their growth period are compared with cultures irradiated from the beginning (table 5), the results on 2 per cent potato-dextrose agar show a mean length of 38.3 and 37.3 microns, respectively, and on cooked potato a mean length of 36.4 and 35.9, respectively. It appears from these results that darkness during the first two days, when growth is beginning and is mostly of a vegetative nature, has little influence on the length of the conidia. If on the other hand, cultures on 2 per cent potato-dextrose agar were kept in the dark for the first four days and thereafter irradiated, the mean length of the conidia in comparison with similar cultures exposed to light for the first four days and then wrapped in black paper for the remainder of the growth period were 29.9 and 39.7 microns, respectively (table 5). When exposed to the light during the entire period, the mean spore-length was 39.4 microns, from which it appears that if light is employed during the first four days, maximum spore size is obtained. On the

Table 5. Frequency distribution of the length measurements of 3-septate conidia of two species of Fusarium grown at 25°C. temperature and artificially irradiated for various portions of the growth period.

	Frequency distribution												
			Fusarium martii var. pisi										
	Cultures on cooked potato					Cultures on 2 per cent potato-dextrose agar							
Length in microns	a	b	a	b	c	đ	e	ť	g	e	g		
22.5					5		1	••					
25.0					41		28			10			
27.5	5	5	2	3	94	1	92	1	4	54	2		
30.0	14	34	9	14	134	8	145	7	15	102	9		
32.5	44	50	23	29	82	20	94	20	52	87	24		
35.0	82	69	44	58	33	47	32	46	88	35	49		
37.5	69	74	75	95	11	88	8	85	123	12	78		
40.0	70	46	91	67		93		116	84		74		
42.5	13	17	44	24		80		82	23		45		
45.0	3	3	10	8		43		36	10		15		
47.5		2	2	2	·	18		6	1		3		
50.0						2		1			1		
Number of conidia	300	300	300	300	400	400	400	400	400	300	300		
Meansh	36.4	35.9	38.3	37.3	29.9	39.7	30.2	39.4	36.9	31.0	38.3		
$SE_m\ \dots\dots$	±.20	±.22	±.21	±.20	±.15	±.20	±.14	±.18	$\pm .17$	$\pm .16$	±.22		
C.V.1	9.4	10.7	9.5	9.5	10.3	10.2	9.4	9.4	9.4	9.2	10.0		

<sup>\*</sup> Dark first 2 days, thereafter irradiated. b Irradiated from the beginning. c Dark first 4 days, thereafter irradiated. d First 4 days light, thereafter dark. Wrapped continuously in black paper. Irradiated during entire period. Twelve hours light; 12 hours dark; alternating during entire period. Expressed in microns. Expressed in percentage.

other hand if light is excluded during the first four days of the growth cycle and then restored for the remainder of the time, the mean length of the conidia is practically the same as in cultures grown during the entire period in darkness, that is, 29.9

and 30.2, respectively (table 5).

Artificial light of the kind and intensity used in these experiments seems to be a very good substitute for daylight. All the species tested behaved in about the same way as when daylight was the source of irradiation, in that the mean spore length was greatest when the cultures were exposed to illumination and shortest when the light was excluded by wrapping the culture in black paper.

Discussion.—The several tables show that the 3-septate conidia of three species of Fusarium attain a greater mean length when the cultures are exposed to either daylight or to artificial irradiation than when they are kept in the dark, but otherwise under identical conditions. Spore length has been used as one of the morphological characters in the delimitation of the different species of Fusarium, and inasmuch as light influences the size of the conidia perhaps more than does the composition of the substratum or the temperature at which the culture is grown, it appears that much of the failure to identify properly some of the species is due in part at least to the influence of light.

In any taxonomic study of species of Fusarium, the ideals sought seem to be, among other things: (1) to establish constancy and uniformity of growth behavior; (2) to obtain copious spore masses in the form of sporodochia or pionnotes, in those species where sporodochia or pionnotes are produced; (3) to obtain maximum size of macroconidia; (4) to secure the largest possible number of septations of the macroconidia. The microconidia and chlamydospores also have characters of taxonomic importance. If the above hypotheses are accepted, any cultural manipulation or external influence such as light or temperature that affects the size or other morphological characters of the conidia must be carefully considered in the identification of the different species. In table 1, Fusarium bulbigenum var. batatas grown on cornmeal agar in the light and in the dark produced spores of a mean length of 38.7 and 31.6 microns, respectively, a difference of 7.1 microns. On green bean pods, in the light and in the dark, the same species produced 3-septate macroconidia of 36.7 and 32.0 microns, respectively, or a difference of 4.7 microns. Wollenweber and Reinking (1935) in the subsection "Constrictum" have separated F. bulbigenum var. batatas and F. bulbigenum var. lycopersici by differences of 1 micron in length and 0.1 to 0.2 microns in diameter, together with slight differences in cultural characters. Fusarium bulbigenum var. tracheiphilum and F. bulbigenum var. blasticola have been separated into two species on the basis of a difference of 2.0 microns in length and of 0.3 microns in the diameter of the 3-septate conidia. There is little doubt that if the longer-spored species were grown in the dark even

for as short a time as 4 days, and the shorter one in the light, the relative lengths of the two species would be reversed and would show far greater differences than those given by Wollenweber and Reinking (1935, p. 107). Other species of this subsection and other subsections of the section Elegans are delimited by equally small differences in the size of the conidia. In many cases such differences are less than would be found in two cultures grown under identical conditions.

The data (table 2) obtained with Fusarium coeruleum grown on different media in the light and dark at room temperature show differences in mean spore length of the 3-septate conidia as follows: cornmeal agar, 8.4 microns; cooked green bean pods, 12.7 microns; 2 per cent potato-dextrose agar, 11.1 microns; cooked potato, 6.1 microns. By the standards employed by Wollenweber and Reinking, and others, these variations probably would be more than enough to justify the creation of a new species irrespective of any other characteristic differences. Such differences are further illustrated by reference to table 3, which shows that F. martii var. pisi follows the same general trend as F. coeruleum on the different culture media. The differences in mean spore-length between cultures grown in the light and those grown in the dark far exceed the differences that are employed to delimit a number of the species of the section Martiella according to the scheme of classification already cited. A study of the data contained in tables 4 and 5 will show that a similar trend is followed by all three species.

That the kind of culture medium used influences the size of the conidia has been demonstrated by various investigators. Sherbakoff (1915) describes a number of species of Fusarium in which spore measurements on different culture media are recorded. The extreme dimensions and average length and width were found to vary according to the substratum. Jones (1923) first studied and described F. martii var. pisi, and his published account of the root rot caused by it records the results of his spore measurements on three different culture media. His results show only an inconsequential variation in average size but a somewhat greater variation in maxima and minima. It is interesting in this connection to note that while Jones and the present writer used one culture medium (2 per cent potatodextrose agar) in common, there was no close agreement in the average length of the 3-septate conidia, which shows that two investigators working with a species of Fusarium of the same line may obtain measurements greater than those that exist between recognized species.

While these investigations were concerned mostly with the influence of light on the length of the conidia, certain physiological characteristics of the organisms were manifest throughout. They produced copious pionnotes on all the culture media in the light and to a lesser extent in the dark. They were all consistent in that the amount of pionnotes was less in the dark and first appeared about two days later than in the light. Under the conditions of these experiments, the mycelial or vegetative growth developed more abundantly in the dark (fig. 1) and seemingly at the expense of fructification. In all cases the 1-celled conidia were more numerous when the cultures were grown in the dark than when they were grown in the light. The 0-, 1-, and 2-septate conidia predominated over the 3-septate conidia as much as 4 or 5 to 1 in the dark, and in some cultures it was difficult to find enough 3-septate conidia for a sampling, while in others there were none. This kind of behavior in the dark was so consistent in all the cultures as to leave no doubt of the definite influence exercised by the light rays emanating either from the sun or from an electric lamp. These data cannot be without significance.

There are probably still other good reasons why workers with species of Fusarium find it difficult to identify them. It is not possible in many cases to determine from a publication whether the cultures were grown partly or entirely in the dark, in subdued light, or in strong light. The temperature or temperatures at which they were grown and what culture media were used is frequently not given. One of the conclusions to be drawn from these investigations is that there should be some standardization of methods of study. The nearest approach to anything resembling directions or methods of procedure is contained in a short account by Wollenweber, Sherbakoff, Reinking, Johann and Bailey (1925) on the production of the norm. Their directions are not specific and leave much to be desired. Most of the discussion centers about the culture media and certain suggestions as to the kind to be used. It is recommended that consideration be given to temperature, humidity and light, but here again nothing is said specifically as to the nature of the consideration to be given. Inferentially it is hinted that light is important only to the extent that color production is usually attributed to its influence.

The influence of light has not received the same attention with reference to the development of fungi that it has for the higher plants, because of the fact that light is absolutely necessary to chlorophyllbearing plants, but not indispensable in the life of fungi. The Fusaria will survive when grown in very dark situations. While light perhaps is not indispensable to the existence of fungi, investigations besides those previously described in this paper have shown that it has a significant influence. With quite a different group of fungi, Yarwood (1937) demonstrated that the sporulation of the downy mildews of hop, onion, grape, and lettuce was more luxuriant at night or under dark conditions than in the light. Similar observations were made by Oltarzhevskii (1935) and by Arens (1929), but contrary results were obtained by Melhus, Van Haltern, and Bliss (1928), Weston (1924) and Hiura (1935) working with different parasites, from which situation it is seen that there is no general agreement in the behavior of the different organisms as regards sporulation, and that no general and sweeping conclusions can be drawn for all fungi from the results obtained by a study of a few species. Each organism must be studied separately.

These experiments were designed to determine if light influences the length of the conidia sufficiently to be significant from both the statistical and the taxonomic points of view. The size of the conidia has almost universally been employed in classifying or separating closely related species with each investigator largely deciding for himself if the differences in their size are important and assigning the organism to a particular species according to his own judgment.

The wide difference in mean spore-length of a single species, the cultures of which were grown under identical conditions except with respect to light, the writer believes would generally be admitted to be sufficiently great to justify the creation of a new species. As might be expected statistically, significant differences of easily measurable and recognizable magnitudes are found in many of the individual groups, which indicate that mathematical calculations of significant differences may have a place in the taxonomic study of certain fungi. To be useful, however, the methods of culture manipulation should be standardized.

It is interesting to note that the different light and other cultural conditions, although markedly affecting mean spore length, had but little effect on the coefficient of variability.3 In most instances, in these studies, the C.V. of a single measurement was about 10 per cent of the mean, but varied from extremes of 8 up to 12 per cent. Assuming a C.V. of 10 per cent, the SEm of 100 measurements at random will be 1 per cent of the mean. Among these populations a minimum of about 100 measurements of each must be made to discover significant differences as small as 1 micron.

Another factor to be taken into consideration where species of the genus Fusarium are separated on the basis of small differences in spore size is the variation among different workers. In order to determine the variation in spore measurements due to the individual, a test was conducted with nine experienced investigators familiar with the technique of measuring conidia. A thin sowing of conidia of a species of Fusarium was prepared on a glass slide in a very weak solution of lactic acid, and after removing the excess liquid, the cover glass was sealed around the edge with paraffin to prevent evaporation. The use of lactic acid extended the time the preparation could be safely used before any plasmolysis or other changes in the conidia took place. Each participant was instructed to measure the length of 100 3-septate conidia to the nearest line on the eyepiece micrometer. The measurements were made consecutively.

<sup>3</sup> Grateful acknowledgment is made to Dr. V. R. Boswell for assistance in the statistical treatment of the figures.

Table 6. Frequency distribution of the length measurements of 3-septate conidia of Fusarium martii var. pisi made by nine investigators.

				Frequer	cy distri	bution			
				Pa	rticipant	S			
Length in microns	A	В	C	D	Е	F	G	Н	I
25.00		1				•••	• •		٠
27.50	1		• • •			• •		•••	
30.00	3	1	2	3	1	1	4		• •
32.50	5	3	3	. • 5	5	2	4	1	
35.00	6	10	14	6	6	9	13	15	6
37.50	23	29	22	16	12	22	29	24	16
40.00	45	24	31	43	37	35	28	29	49
42.50	16	21	22	19	30	25	16	22	15
45.00	1	8	5	8	12	5	6	9	9
47.50		3	1			1			5
Number of conidia	100	100	100	100	100	100	100	100	100
Mean	38.8	39.4	39.2	39.5	40.5	39.7	38.6	39.6	40.5
SE <sub>m</sub>	±.31	$\pm .37$	±.11	$\pm .11$	$\pm .30$	±.30	$\pm .34$	$\pm .30$	±.29
C.V.a	8.0	9.4	2.8	2.8	7.5	7.5	8.9	7.6	7.1
Chi-sq.b	15.1	7.6	3.4	6.6	12.2	2.2	10.0	8.0	18.2

<sup>\*</sup>Expressed in percentage. <sup>b</sup> For 5 degrees of freedom 5 per cent point=11.1; for 1 per cent point 15.1.

The data submitted by the different participants are shown in table 6, in which the frequency distribution of spores is arranged in micron classes.

An examination of the data of table 6 shows that the mean length between extremes is only 1.9 microns (A, I, and E), a difference that some mycologists might not consider large enough to be determinative of different species. The differences between the mean lengths obtained by other participants are still less. It is interesting to note also that the mode of 7 out of 9 sets of measurements fall in the 40 micron class and two in the 37.5. There is considerable difference, however, between some of the sets in the maximum ranges. For minimum sizes one participant (B) reports one conidium in the 25-micron class and one (A) in the 27.5 class, while the shortest measurement made by one individual (H) was in the 32.5 micron class, and another participant (I) measured no conidia shorter than 35 microns in length, of which there were six. The range in microns covered by B on the one hand and H and I on the other, represents quite a variation, although the mean length between the two sets is less than one micron. The difference in length of the smallest conidia in these two classes, however, is 10 microns, while the average length differs by less than one micron.

A statistical treatment of these data shows significant differences between the results obtained by some of the participants, the extremes being represented by G and I. On the other hand there is no satistically significant difference between measurements made by A and B and those made by some of the other participants. Nevertheless the indications are that on the basis of the results in table 6, the

participants would not have agreed on the species represented by the culture from which the conidia were taken.

Inasmuch as statistically significant differences occur in the spore measurements of 9 investigators made from the same preparation, even greater differences are to be expected where the cultures of the organisms are exposed to different environmental conditions.

The variations that occur among the different participants may have been due to several causes, among which may be mentioned: (1) unconscious selection of large, medium, or small conidia; (2) measuring conidia in different fields of the spore preparation; and (3) error in judgment as to the nearest line on the scale of the eyepiece micrometer. Any one factor or a combination of these factors probably could cause the differences indicated in table 6.

#### SUMMARY

The effect of sunlight and artificial irradiation on the length of the 3-septate conidia was studied for three species of *Fusarium*, grown on several different culture media.

The conidia were considerably longer in cultures grown in the light than in those grown in the dark. Cultures exposed to sunlight for 3 hours daily at room temperature produced spores much longer than did cultures grown in the dark.

The exposure (light or dark) of the cultures for the first two days after inoculation had little effect. Cultures held in the dark for the first two days and then irradiated for the remainder of the growth period produced spores about equal in length to those exposed to light from the beginning. If, on the other hand, the culture was incubated in the dark for the first 4 days following inoculation, the results were about the same as when the cultures were kept in the dark during the entire incubation period. If, however, the cultures are irradiated for the first 4 days following inoculation and then shifted to a dark situation, the results were about the same as when they are grown in the light during the entire growth period.

Sporodochia and pionnotes were more copious in cultures in the light than in those grown in the dark.

Vegetative growth was most abundant in the dark. The difference in length of spores from cultures grown in light and in dark under otherwise identical conditions was much greater than the difference used as a means of separating certain species.

The maximum number of macroconidia occur in the light while microconidia predominate in the dark.

Cultures incubated in the light generally give more abundant sporodochia or pionnotes and produce larger and more numerous macroconidia than those grown in the dark.

Statistically significant differences occurred when different investigators measured conidia from the same microscopic preparation.

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## ORGANIC ACID CONTENT OF CORN PLANTS AS INFLUENCED BY PH OF SUBSTRATE AND FORM OF NITROGEN SUPPLIED 1

Cecil H. Wadleigh and John W. Shive

THE PRONOUNCED influence of the presence of ammonium nitrogen in the growth substrate upon the general appearance and the mineral composition of corn plants grown in sand culture has been pointed out in previous papers (Wadleigh, Robbins, and Beckenbach, 1937; Wadleigh and Shive, 1939). This was especially manifested by the almost complete absence of chlorosis and by the significantly lower base content found in the plants supplied with a culture solution containing both ammonium and nitrate nitrogen in approximately equivalent proportions than in those supplied with nitrate as the sole source of nitrogen. It was also pointed out (Wadleigh, Robbins, and Beckenbach, 1937) that those plants supplied with nitrogen in the form of a cation had an appreciably lower pH of the expressed sap than had those supplied with nitrate as the sole source of nitrogen. In the previous paper, evidence concerning a relationship between the pH and iron solubility of a culture medium was presented together with a consideration of the probability that iron availability within the tissues may have a direct bearing upon chlorotic intensity.

The works of Harrar and Germann (1931, 1932) and of Smythe and Schmidt (1930), showing the greater solubility of organic forms of iron over inorganic forms in neutral or alkaline media, suggested the possibility that the organic acid anions present in the plant might have some bearing on the solubility of iron within the plant. The present work was undertaken to investigate the possibility that iron availability in the plants may be influenced by amounts of certain organic acids present in the plant.

The extensive studies of Pucher and Vickery and their associates have greatly simplified and improved the procedure of analyzing plant tissue for small quantities of certain organic acids. Their methods (Pucher, Vickery, and Wakeman, 1934a,

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1934b; Pucher, Vickery, and Leavenworth, 1934) make possible the detection of oxalic, malic, and citric acids, and they provide a means of determining total titratable organic acids. Tartaric acid was determined by the method of Underhill et al. (1931). These acids are by no means the only ones existing in plants. Succinic, malic, fumaric, glutaric, glycolic, and others are to be found in various species of plants in varying amounts. The chemical similarity of many of these acids mitigates against a satisfactory assay when present in relatively small quantities. However, Pucher and his associates were able to account for 80 per cent of the acidity in the leaves of the tobacco plant by determinations for oxalic, citric, and malic acids.

In the present study, the tissues used for the organic acid analyses were parts of the same plants which were considered in the previous studies (Wadleigh, Robbins, and Beckenbach, 1937; Wadleigh and Shive, 1939). It is needless, therefore, to repeat the description of the technique used in growing them.

RESULTS AND DISCUSSION .- Plants receiving nitrate nitrogen only.-Repeated tests failed to reveal the presence of tartaric acids in sufficient quantities for detection. The results of the analyses for oxalic, malic, citric, and total acids are shown in table 1. The total organic acid content of these corn plants varied from 0.79 milli-equivalents per gram dry weight at pH 4.0 to 0.98 milli-equivalents per gram dry weight for plants grown at pH 8.0. There was a marked tendency for total organic acid to increase with increase in pH of the growth medium. However, the plants grown at pH 3.0 showed a higher total organic acid content than did the plants grown at pH 4.0 or 5.0. It is of interest to compare the values herein reported for corn with those reported by Pucher et al. (1934a) for tobacco leaves. They found an organic acid content of from 1.6 to 2.4 milli-equivalent, per gram dry weights which represents from two to three times the corresponding values for corn plants.

Table 1. Organic acid content of corn plants grown in sand supplied with nitrate as the sole source of nitrogen.

pH of sub- strate	Total organic acids, M.E. per gram	Oxalic acid, per- centage dry wt.		Malic acid, per- centage dry wt.	Malic acid, M.E. per gram	Citric acid, per- centage dry wt.	Citric acid, M.E. per gram	Acids unaccounted for M.E. per gram
3.0	0.842	0.062	0.014	1.25	0.187	0.130	.020	0.621
4.0	0.789	0.036	0.008	1.42	0.212	0.12	.019	0.550
5.0	0.808	0.054	0.012	1.56	0.233	0.18	.028	0.525
6.0	0.956	0.145	0.032	1.69	0.252	0.23	.036	0.636
7.0	0.956	0.196	0.043	1.83	0.273	0.24	.038	0.602
8.0	0.984	0.178	0.040	1.72	0.258	0.27	.043	0.643

The oxalic acid content of the plants varied considerably, ranging from 0.036 per cent in plants grown at pH 4.0 to 0.196 per cent in those grown at pH 7.0. This acid tended to increase in plants grown at the higher pH levels, but the amount found in the plants of any treatment was rather low and made up only a very small portion of the titratable organic anions of corn. Citric acid was also found to be rather low in these corn plants, varying from 0.12 in plants grown at pH 4.0 to 0.27 per cent in those grown at pH 8.0. This acid likewise was higher in amount in the plants grown at the lower H-ion concentrations than in those grown at the higher H-ion concentrations. However, it accounted for only about 2 per cent of the titratable organic anions in corn, and furthermore, this low value contrasts markedly with the value of 2.8 to 3.0 per cent found by Pucher et al. (1934) in the leaves of the tobacco plant. Malic acid was found in appreciable quantities in these corn plants, ranging from 1.25 per cent to 1.83 per cent in plants grown at pH levels of 3.0 and 7.0, respectively, with a consistent increase in amount present in the plants with progressively higher pH values of the media in which the plants were grown. About 25 per cent of the total organic acid could be accounted for by the malic acid content. Although the values recorded in table 1 might indicate that the corn plant contains a rather large quantity of malic acid, it should be pointed out that Pucher et al. (1934b) found from 4 to 9 per cent malic acid in tobacco leaves.

A significant point brought out by the data in table 1 is the large proportion of titratable organic acids which could not be accounted for by the individual analyses. That is, the specific acids determined account for only about 30 per cent of the total organic acid anions in corn, whereas Pucher and his coworkers were able to account for about 75 per cent of the total organic acids in the tobacco plant.

It appears logical that the increased accumulations of organic anions in the plant grown at progressively higher pH levels might be due to increased accumulations of absorbed cations, which, on combining with the organic anions and possibly precipitating them, would render them less susceptible to further oxidation. However, a corresponding increase in base content of the plants grown at pro-

gressively higher pH levels was not found. In fact, the base content of plants of all treatments, excepting those grown at pH 3.0 was practically identical. Furthermore, the plants grown at pH 3.0, which contained an appreciably lower quantity of total base than did the plants grown at higher pH levels, were found to contain a higher quantity of total organic acids than the plants grown at pH 4.0 or pH 5.0. In view of the rather complete absence of a correlation between these components, it should be made clear that the methods for determining organic acids detect not only the free acids but also salts and esters of these organic acids, and all of these form a composite sample. The total base content values are also subject to similar stipulations. Thus, even though very pronounced relationships might have existed between the organic acid content and the bases present in certain corresponding tissues of the plants grown at the various pH levels, such relationships might easily have been masked by the composite sampling used and the method of analyses employed.

At first glance, it might appear that the data of table 2 show little correlation between "soluble" iron and organic acids in the plants grown at different pH levels. However, it may be of some significance that the plants grown at pH 4.0 were lowest in both soluble iron and organic acids. Furthermore, the plants grown at pH 3.0, which did not follow the general tendency with respect to the relation between organic acid content and pH of the growth medium and which contained a higher percentage of these acid anions than did the plants grown at either pH 4.0 or pH 5.0, showed the highest "soluble" iron (Ingalls and Shive, 1931) content. The plants grown at pH 8.0, which showed a relatively high soluble iron content and which were free from chlorosis, contained the highest percentage of organic acids.

On the whole, there appears to be no significant correlation between pH of the expressed sap and the organic acid content of the plants. This is not surprising, since, as has been pointed out many times, there is little correlation between total and active acidity of plant juices due to the divers buffering substances present.

<sup>2</sup> The methods for determining total and "soluble" iron were those used by Ingalls and Shive (1931).

Table 2. Organic acids, total bases, soluble iron, and titratable acidity of the expressed sap of plants supplied with nitrate as the sole source of nitrogen.

pH of sub- strate	Total organic acids, M.E. per gram	Oxalic acid, M.E. per gram	Citric acid, M.E. per gram	Malic acid, M.E. per gram	Total bases, M.E. per gram	"Soluble" iron, percentage	pH of expressed sap	Titratable acidity of sap, M.E. per gram
3.0	0.842	0.014	0.13	0.187	2.06	.00171	5.56	0.542
4.0	0.789	0.008	0.12	0.212	2.47	.00096	5.73	0.414
5.0	0.808	0.012	0.18	0.233	2.50	.00136	5.70	0.420
6.0	0.936	0.032	0.23	0.252	2.52	.00114	5.72	0.466
7.0	0.956	0.043	0.24	0.273	2.50	.00108	5.74	0.472
8.0	0.984	0.040	0.27	0.258	2.43	.00132	5.70	0.362

Table 3. Organic acid content of corn plants grown in sand supplied with both nitrate and ammonium nitrogen in approximately equivalent proportions.

pH of sub- strate	Total organic acids, M.E. per gram	Oxalic acid, per- centage dry wt.	Oxalic acid, M.E. per gram	-	Malic acid, M.E. per gram	Citric acid, per- centage dry wt.	Citric acid, M.E. per gram	Acids unaccounted for M.E. per gram
3.0	0.725	0.066	.015	0.76	0.113	0.04	.006	0.591
4.0	0.780	0.128	.028	1.22	0.182	0.13	.020	0.550
5.0	0.805	0.168	.037	1.33	0.199	0.12	.019	0.550
6.0	0.855	0.184	.041	1.41	0.211	0.16	.024	0.579
7.0	0.895	0.176	.039	1.52	0.227	0.19	.029	0.600
8.0	0.905	0.188	.042	1.49	0.222	0.21	.032	0.609

Plants receiving both nitrate and ammonium nitrogen.—In addition to the lower base content observed in the plants grown in a substrate containing both ammonium and nitrate nitrogen than in those grown with nitrate alone as the souce of nitrogen, it was interesting to note also that the organic acids were present in the plants of this series in somewhat smaller amounts than in those of corresponding cultures of the preceding series. Table 3 presents the total organic acids, oxalic acid, malic acid, and citric acid found in the plants of this series. Total organic acids varied from 0.725 to 0.905 M.E. per gram of dry plant substance, the respective amounts varying directly with the pH of the substrate. Oxalic acid varied between the limits of 0.066 per cent and 0.188 per cent, with a consistent increase in amount as the H-ion concentration of the growth medium was decreased. Malic acid varied from 0.76 per cent to 0.152 per cent and citric acid from 0.04 to 0.21 per cent, each showing the same direct correlation with pH of the substrate as did the oxalic acid content. Almost without exception, all the organic acids were found to be lower in the plants of this series than in those of the corresponding cultures grown with nitrate as the sole source of nitrogen. It is especially outstanding that the plants grown at pH 3.0 contained, relatively speaking, very low amounts of these anions. Thus the data of table 3 indicate a definite relation between organic acid content of the plants and pH of the growth substrate, high organic acid content corresponding to high pH values of the growth substrate, within the limits here investigated, and low acid content corresponding with low pH values of the substrate.

A satisfactory explanation of this relationship is at present not forthcoming. It is known that organic acids are continually being formed in plants as a result of proteolysis and oxidation of sugars, and that at least some of these organic acids are themselves continually being further decomposed by oxidation. If an organic acid should form an insoluble salt with a given cation, it should become resistant to further oxidation and should tend to accumulate in the plant. Thus, any condition favoring the precipitation of the organic anions should favor the accumulation of these anions.

It appears from the data of table 4 that there is a direct correlation between the pH of the expressed sap and the accumulation of organic acids. The significance of this relationship is emphasized by the fact that both the pH of the expressed sap and the organic acid content of the plants of the preceding series showed higher values than they did in the plants of the corresponding cultures of the series here considered. Titratable acidity of the expressed sap appears to vary inversely with the content of organic acids, as indicated by the data of table 4. This is not surprising, since, as already indicated, the variations in titratable acidity could, for the most part, be accounted for by variations in phosphorus content of the expressed sap. This comparison of the variations in titratable acidity of the ex-

Table 4. Organic acids, total bases, soluble iron, and titratable acidity of the expressed sap of plants grown in sand supplied with both nitrate and ammonium nitrogen in approximately equivalent proportions.

pH of sub- strate	Total organic acids, M.E. per gram	Oxalic acid, M.E. per gram	Citric acid, M.E. per gram	Malic acid, M.E. per gram	Total bases, M.E. per gram	"Soluble" iron, percentage	pH of expressed sap	Titratable acidity of sap, M.E. per gram
3.0	0.725	0.015	.006	0.113	1,61	.00110	5.44	0.555
4.0	0.780	0.028	.020	0.182	2.02	.00124	5.46	0.469
5.0	0.805	0.037	.019	0.199	2.16	.00148	5.46	0.505
6.0	0.855	0.041	.024	0.211	2.15	.00155	5.51	0.449
7.0	0.895	0.039	.029	0.227	2.09	.00135	5.60	0.416
8.0	0.905	0.042	.032	0.222	1.94	.00123	5.64	0.362

pressed sap with variations in organic acid content further suggests that the accumulation of organic acids may be due to precipitation, since their accumulation did not affect the buffer capacity of the expressed sap.

There was no marked relationship between the variations in organic acids and total bases. It is quite evident, however, that the plants grown at pH 3.0 showed by far the lowest values for both these components. As previously pointed out, both these constituents were lower in the plants of this series than in the plants of corresponding cultures of the

preceding series.

As previously stated in presenting the results of the preceding series, little correlation was observed between the soluble iron content and the organic acids present in the plants grown with nitrate as the sole source of nitrogen. The data of table 4, on the other hand, indicate a direct relation between the soluble iron content and the organic acids in the plants grown with both ammonium and nitrate as the sources of nitrogen, within a pH range of the substrate extending from pH 3.0 to 6.0.

The results of several investigators (Harrar and Germann, 1931, 1932; Smythe and Schmidt, 1930) would lead to the anticipation of such a relationship, and while it is quite possible that the present observation may carry some significance, it might be safer, without further investigation, to regard it as

merely fortuitous.

In this connection it should be emphasized that the soluble iron content was higher and the organic acid values lower in the plants of cultures in which nitrate was the sole source of nitrogen than they were, respectively, in the plants of corresponding cultures in which both ammonium and nitrate nitrogen were present. This observation opposes the above relation.

That a more definite correlation was not observed between soluble iron content and the organic acids present may be accounted for by the fact that the methods of analysis do not differentiate between soluble and precipitated organic anions in the plants. It is interesting to note, however, that the corn plant shows a relatively low content of organic acids and that it displays a correspondingly low degree of

efficiency in the utilization of its iron.

The large proportion of unidentified organic acids in the corn plant naturally gives rise to the questions of the nature and composition of these unidentified acids and their probable significance. Unfortunately, these questions can not be definitely answered. Yet, without going into the lengthy mathematical procedure, it might well be mentioned that from the titration curves of the extracted juice the value dpH/dB (Small, 1929) was determined by drawing a tangent to the curve at the point corresponding to the pH of the expressed juice. The phosphate content and the malic, oxalic, and citric acid content of the extracted juice were known as was also the pH of the expressed juice which had a value

of pH 5.5 to 5.7. Since the pK1 and pK2 values of phosphoric acid are 2.10 and 7.21 (Small, 1929), respectively, it is apparent that the expressed juice of the plant is buffered at a pH at which phosphates have a comparatively low buffer value. Calculations revealed that the phosphate content (which in these plants was relatively high) of the corn plants could account for only 15-20 per cent of the buffer activity at the pH of the expressed juice. Likewise, malic acid, with a pK2 value of 5.11 becomes relatively low in buffer activity at a pH value of 5.6 to 5.7. This relation has been illustrated and discussed by Small (1929). The malic acid content of these plants could account for only 10-15 per cent of the buffer activity observed at the pH value of expressed sap. Succinic acid has a pK2 value of 5.6, indicating that it could be a very active buffer in expressed corn sap at the pH of the sap. By assuming that the unaccounted for organic acids might be primarily succinic acid, and by taking into account the malic and phosphoric acid present, it was possible to calculate a value for dpH/dB at the pH of expressed sap which was strikingly close to the observed value. However, a satisfactory method for determining small quantities of succinic acid was not at hand, and, consequently, no definite statement can be made relative to the actual presence of succinic acid. It is apparent, however, that few other substances common to plants have the physical characteristics which would fit the experimental observations.

### SUMMARY

Oxalic, malic, citric, and total organic acids were determined in corn plants grown at various pH levels, the cultures of one series being supplied with nitrate nitrogen only and those of the other with both nitrate and ammonium nitrogen.

The total organic acid content of corn plants varied between 0.7 and 1.0 milli-equivalents per

gram dry weight.

Oxalic and citric acids were present in small amounts, varying from 0.05 to 0.27 per cent of dry weight.

Malic acid was present in appreciable quantities, varying from 0.8 to 1.8 per cent of dry weight.

Only 25-30 per cent of the total organic anions were accounted for.

The organic acid content of the corn plants increased with increase in pH of the substrate.

Plants grown with ammonium and nitrate nitrogen contained less organic acids and had a lower base content than plants grown with nitrate as the sole source of nitrogen.

The data secured do not show any definite interrelationship between soluble iron content and organic acids in corn plants. Corn plants under treatments here considered were relatively low in both organic acids and soluble iron.

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### THE EMBRYOGENY OF SEQUOIA SEMPERVIRENS WITH A COMPARISON OF THE SEQUOIAS <sup>1</sup>

### J. T. Buchholz

THE REDWOOD, Sequoia sempervirens, has been investigated embryologically by Shaw (1896), Arnoldi (1899, 1900), Lawson (1904), and Looby and Doyle (1937). Strasburger (1872, 1879) described only the cone, ovules, and vascular supply of the cone scale. There are a number of points of disagreement in the earlier embryological descriptions. Although Looby and Doyle cleared up many disputed features concerning the gametophytes and fertilization, their account, in so far as it pertains to the embryo, is inadequate, except for the proembryo stages. While in California in 1936 the writer obtained a series of stages of the gametophytes before and including fertilization and made a careful study of the embryogeny for purposes of comparison with S. gigantea. Because I am using my own figures in this paper, it is necessary to repeat a number of facts and details concerning the gametophytes which were previously observed by others, and there are still some uncertainties which await further investigation.

A relatively small tree about 40 feet high in a group found in cultivation on the grounds of Stanford University was selected for this investigation. Although some cones on this tree were within reach, it was soon found that those situated high on the tree were not only larger, but also better pollinated. A specially constructed bamboo pole tipped with a snare was used to reach cones up to 30 feet from the ground, where they were very abundant. These

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high ones were found to have about 60 per cent good ovules, while the lower group had less than 30 per cent good ovules. For this reason nearly all the material used for sectioning and dissection came from cones situated high up on this tree.

Additional material was collected from the Palo Colorado Canyon south of Carmel. This is in the cooler fog belt, and the stages observed were not as advanced. In this location, where the Redwood is found in a native stand but near the southern extremity of its region of distribution, there were very few healthy seeds in the cones that could be reached, but there were many cones high up in the trees. The cultivated trees at Stanford University in the Santa Clara valley were found to be much more productive of good seeds than any other location which was visited in this more southern region. Since Lawson collected his material at Palo Alto, it seemed desirable to obtain the material for a reinvestigation of this species from the same source, but the dates for my stages are about a month earlier than the dates given by Lawson for corresponding stages. On the other hand, the dates given for fertilization and the various stages of the embryogeny might be considerably later than those given by Lawson if the cones were obtained in the native region, either farther north or bordering the cool California coast at the same latitude. The material was killed and sectioned by the same methods as for the Big tree (Buchholz, 1939).

Figures 1 and 2 represent tranverse sections of an exceptionally large nucellus and female gametophyte. In figure 1 slightly above the middle, many archegonia or enlarged archegonial initials are

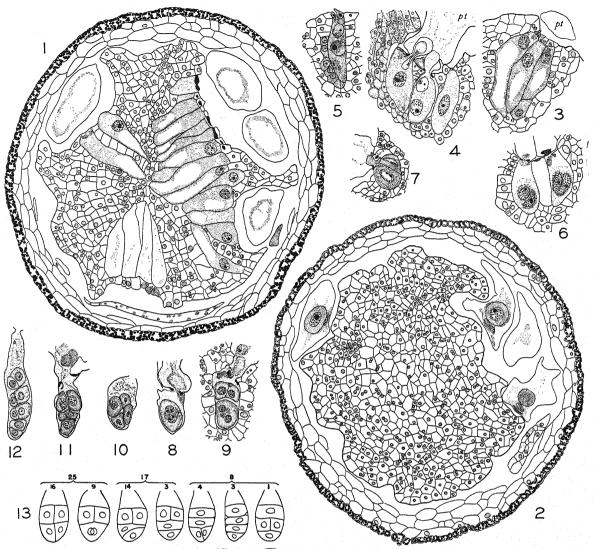


Fig. 1-13.—Fig. 1. Transverse section through upper half of nucellus of Sequoia sempervirens containing female gametophyte with many archegonia adjacent to 4 pollen tubes. A second female gametophyte may be seen below, in free nuclear stage, crushed against nucellus. May 20. ×100.—Fig. 2. Transverse section below middle of female gametophyte and below the region of archegonia (same ovule as fig. 32). Three of the pollen tubes show body cells and one or both of the tube and stalk nuclei. Part of a crushed third female gametophyte is shown at the lower right.  $\times$ 100.—Fig. 3. Archegonial group showing three archegonia near a pollen tube (pt). No ventral canal nucleus could be found. May 24. ×100.—Fig. 4. Fertilization. The archegonium at the extreme right has egg nucleus centrally placed but shows no trace of a ventral nucleus. The other two archegonia are being penetrated by sperms. In the one at the left the top of the egg is covered by dense cytoplasm which came from the sperm. The nucleus in the act of entering is shaped like an hour glass, with nucleolus likewise elongated. In the other archegonium the male nucleus has entered, leaving most of its cytoplasm outside in the pollen tube. Note that the male nucleus appears slightly smaller than the egg nucleus. May 27. ×100.—Fig. 5. Fertilization stage similar to most advanced stage in fig. 35 to show comparative size of male and egg nuclei. This archegonium shows a group of large deeply-staining jacket cells at the left. May 27. ×100.—Fig. 6. Fertilization of two archegonia with another archegonium between. The unequal sperm and egg nuclei are in close contact and both surrounded by a more deeply staining zone of cytoplasm. These two archegonia were entered by sperms from different pollen tubes. The sperm cytoplasm partly inside and partly outside may be recognized by its deeper staining quality, but this male cytoplasm does not extend to the lower part of the egg where the embryo will be formed. May 26. ×100.—Fig. 7. Two-celled proembryo showing remains of sperm cytoplasm in pollen tube. May 28. ×100.—Fig. 8. Proembryo of S. sempervirens, showing wall formation after the first division of the zygote. May 28. ×100.—Fig. 9. Proembryo forming walls after the second division, resulting in 4 cells. May 29. ×100.—Fig. 10, 11. Four-celled proembryos with different arrangement of cells.—Fig. 12. Proembryo with 4 embryo units arranged in a row, with each unit already divided into a smaller embryonic cell and a primary suspensor cell. May 29. ×100.—Fig. 13. Diagrams to show frequency of distribution of variations in cell arrangement in fifty 4-celled proembryos which were carefully examined.

shown, and these continue to be numerous toward the micropylar end in nearly all sections. Some of these archegonia may be fully developed, but most of them have not reached the mature stage. In sections at this level and upward toward the tip of the nucellus, the archegonia may be in the one-celled archegonial initial stage, or they may have formed the primary neck cell; sometimes the latter has divided. Smaller gametophytes have smaller numbers of archegonia, especially if fewer pollen tubes are present. In comparison with the Big tree, the archegonia are not only larger but many times more numerous.

Previous investigators have pointed out that there are usually several female gametophytes present in earlier stages of development and that all but one of these abort. This feature was easily confirmed by dissection. The same thing is shown in many later stages. Figure 1 shows, at the lower margin of the figure, the flattened remains of an aborted female gametophyte which is still in the free-nuclear stage of development, and in the lower right margin of figure 2 is a portion of another which had become cellular. It is interesting to note that the contents of the group of archegonia that lie opposite the first named abortive female gametophyte have disintegrated, while those that lie next to pollen tubes are more or less normally developed. Some of the archegonia shown in figure 1 are still one-celled and represent only enlarged archegonial initials, others have cut off the primary neck cell; in some the neck cell has divided so that archegonial development is nearing completion. However, the archegonia which contain the eggs that are actually fertilized are often not quite so large as many of those shown in figure 1. Furthermore, they do not usually have large vacuoles. Figure 3 shows a typical group of archegonia which are ready for fertilization, but one of these does have a relatively large vacuole. The archegonia in which, or near which, fertilization was observed (fig. 4, 5) were found to have small vacuoles, if any. Eggs that are fertilized are usually situated in archegonia far down near the lowest limits for these structures; several archegonia may be observed only a few sections above the one delineated in figure 2.

As far as could be determined, the archegonia are all related to the surface of the female gametophyte, where neck cells may usually be identified if the archegonia are sufficiently matured. If no pollen tubes are present in an ovule, the archegonia may have a scattered distribution over the surface of the upper half of the gametophyte; if pollen tubes are present, the archegonia are most abundant on the surfaces adjacent to pollen tubes. These archegonia and archegonial initials have a dense cytoplasm and stain more deeply than other gametophytic cells.

Although the origin and development of the archegonia were not studied intensively, it appears that some of them are divided later to form ordinary cells of the female gametophyte, with some of their derivative cells remaining enlarged for a time to

form jacket cells which exist in patches adjacent to the remaining archegonia. This disposition and the abortion of some of them reduces their number. Isolated cells of this kind, when found deeply imbedded, may give a misleading appearance of archegonial initials originating within the tissue. Lawson (1904) reported that the archegonia originate within the tissue. This is probably an error; at least the functional archegonia are peripheral in origin, have cut off and differentiated neck cells next to the surface of origin, and have a definite relation to pollen tubes. Sections through archegonia may appear deep seated because their necks may be at one side rather than at their ends. The archegonium containing a proembryo in figure 7, if cut in a section vertical to the surface of the drawing and in the long axis of this archegonium and embryo, might appear completely surrounded by tissue.

The archegonia are usually grouped close together with adjacent archegonia separated only by a single cell wall. This grouping is similar to the archegonial complex of the Cupressaceae but with the important difference that the entire complex is not surrounded by a common jacket, for in Sequoia we find only scattered jacket cells or small groups of such enlarged cells, if any. Proembryos were found in archegonia with no adjacent jacket cells. Since both gametes in each pollen tube may function, the fertilized eggs or zygotes are usually situated in pairs.

Looby and Doyle have pointed out that the megaspore membrane is much thinner in S. sempervirens than in S. gigantea. This difference was obvious in my studies. Measurements show that this membrane is only slightly more than a half micron thick in the Redwood but 2-layered,  $2-3\frac{1}{2}$  microns thick in the Big tree.

As in the Big tree, the nucellus of the Redwood as well as the female gametophyte which it encloses undergo considerable growth and elongation in their basal regions, even after the fertilization stage, so that the upper portion of the nucellus is soon crowded into the space between the integuments.

Sections of four pollen tubes are shown in figures 1 and 2. In the sections which show archegonia the body cells of the pollen tubes are usually not visible; at the level of the body cells there are frequently no archegonia at all, as shown in figure 2. It appears that when fertilization takes place the male cells must actually move upward toward the micropyle in order to come to the level of the lowest archegonia unless the growth of the nucellus drags the pollen tube upward. The paired proembryos were usually observed in some of the archegonia situated farthest from the micropyle.

The body cell of the pollen tube becomes very large, much larger than the corresponding structure in S. gigantea. Likewise the pollen tubes have a greater diameter. The nucleus of the body cell was observed in mitosis forming an intra-nuclear spindle, which occurs very shortly before fertilization. The male gametes become pear-shaped or more or

less nearly spherical with a pointed end, resembling a tear drop. The pointed ends are presented to the necks of the archegonia at the time of fertilization.

In an examination of many archegonia I found several abnormal instances of large or greatly elongated archegonia with two nuclei, one in which there were three and another with four. These extra nuclei were not caused by fertilization, nor was it certain that they represented the division of the central cell nucleus to form a ventral nucleus and an egg nucleus. These abnormalities may have developed either because walls failed to form in the subdivision of archegonia to form jacket cells or because there was a fusion of neighboring archegonia or archegonial initials. Such abnormal archegonial structures were relatively rare and in positions where eggs were usually not fertilized. After examining thousands of archegonia in stages just before fertilization and at the time of fertilization, Lawson's figures of ventral nuclei become less convincing. Although I observed conditions similar to his figure, my impression is that these are abnormalities and that the ventral nuclei are not regularly formed in Sequoia sempervirens. This is a problem that awaits further investigation.

Many stages in fertilization were observed. A few are illustrated in figures 4, 5, and 6. Figure 3 shows the two male gametes of a pollen tube in the act of entering the eggs of two adjacent archegonia. In the figure at the left some of the male cell cytoplasm may be observed at the top of the egg cytoplasm (staining differently), and the nucleus is squeezing through, showing a nuclear membrane shaped like a dumbbell or hour glass, the nucleolus being likewise elongated. The egg nucleus is centrally located. In the figure at the right (the middle archegonium of this group) the nucleus of the other male cell has entered the top of the egg and the egg nucleus has migrated farther down. Most of the male cell cytoplasm has remained outside, some has entered and shows near the top in another section of the series to which figure 4 belongs. Eventually only the nucleus of the male cell actually contributes to the union of cells. The sexual nuclei migrate to the base of the egg, and the foreign cytoplasm remains at the top near the entrance, so that it appears not to be included in the zygote, which occupies only the lower two-thirds of the archegonium at the time of the first division.

The male nucleus is somewhat smaller than the egg nucleus. This point was carefully checked and is shown in figure 4 (middle archegonium), in figure 5, and in the two zygotes of figure 6. This is a minor detail in which my observations differ from those of Looby and Doyle (1937). Sometimes darkstaining patches appear in the egg cytoplasm, but these are not as conspicuous as the "Hofmeister bodies" in the eggs of pines and many other conifers at the time of fertilization.

After the sexual nuclei are brought into contact, as in figure 6, a denser zone of cytoplasm appears around both of them, a phenomenon of fertilization

reported in other conifers, and the cytoplasm occupying the upper third of the archegonium begins to disorganize.

THE EMBRYO.—The anaphases and telophases of the first division of the zygotic nucleus were observed. The first division is usually transverse to the long axis of the archegonium. Figure 8 shows a very late telophase in which the wall is forming between nuclei that have divided. This is a normal cell division forming a cell plate, as will be seen also by comparing figure 9, where the first two cells have become dissociated. Thus the writer confirms the fact pointed out by previous investigators that in the proembryo of S. sempervirens there is no free-nuclear stage. The proembryo throughout the first two cell divisions occupies about two-thirds of the lower part of the archegonium. Each zygote normally forms a proembryo of 4 cells, each of which is a potential embryo initial, but soon after this 4-celled stage the entire proembryo may enlarge and crowd all adjacent structures.

Figures 7-12 show several of the types of cell arrangement found in different proembryos. Fifty proembryos were examined and classified as nearly as possible into the various categories of cell arrangements with frequencies shown by numerals in the diagrams of figure 13. Thus it may be seen that figures 9 and 10 are the more common type of arrangement and that figures 11 and 12 represent the less common types. Figure 12 shows an 8-celled proembryo in linear arrangement, but here there are really four 2-celled embryo units. Each of the four cells has divided to form an enlarging primary suspensor cell and a smaller embryonic cell. Probably the shape of the archegonium and its position with reference to the pollen tube on the one hand and the axis of the female gametophyte on the other are factors in determining the type of cell arrangement in the proembryo.

Looby and Doyle (1937) have recorded several similar variations in the 4-celled proembryo so that my study confirms their work in its essentials and adds a quantitative estimate of this variation. Otherwise the facts concerning the proembryo given by Arnoldi (1900), Shaw (1896), and Lawson (1904) were so incomplete and fragmentary that a thorough reexamination and description of these stages was fully warranted. Lawson's mitotic figure of the first division of the zygotic nucleus is not convincing. It does not show the disorganizing cytoplasm in the upper third of the archegonium and may represent an archegonial initial or large jacket cell which is dividing to form ordinary cells of the gametophyte.

Looby and Doyle, who reported that they did not observe the division of the zygote, are nevertheless correct in stating that the proembryo of S. sempervirens is four-celled. They did not pretend to give much definite information beyond this stage, and the figures which they gave of older stages were not interpreted correctly. Their text figure 5a seems to represent the formation of a four-celled proembryo,

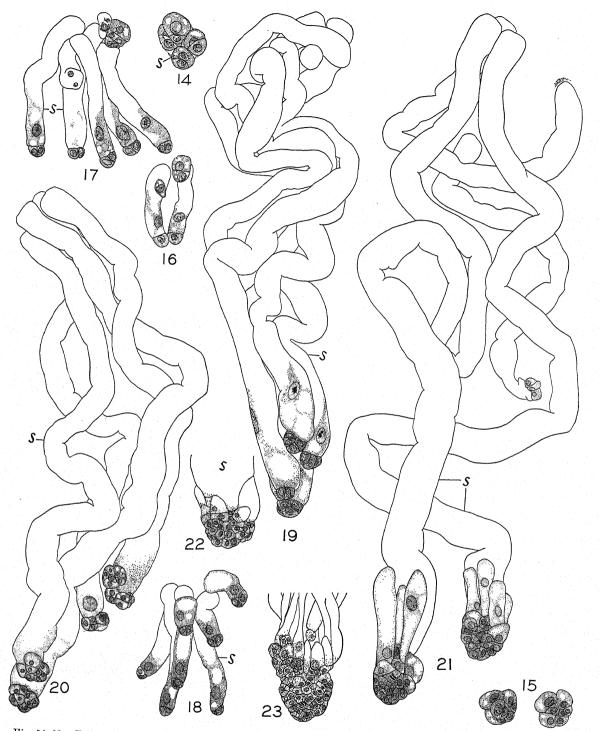


Fig. 14-23.—Fig. 14. Late proembryo from dissected specimen of Sequoia sempervirens showing each of four proembryonic cells divided into (large) primary suspensor cell (s) and (smaller) embryonic cell. May 29, 1936. ×100.— section. One embryo (at the right) has become 3-celled. May 29. ×100.—Fig. 16. Embryo system in early stage of sussuspensors elongation. One embryo initial still undivided, one divided, but with suspensor not elongated; two with fertilization of two closely adjacent archegonia, some of which are aborting to give 6 or 7 embryos. June 1, 1938. ×100.— Fig. 18. Seven embryonic units coming from two neighboring zygotes. June 7, 1936.—Fig. 19. Four embryos from the same zygote with well developed primary suspensors (s). June 8, 1936. ×100.—Fig. 20. Four embryos from the same

but 5d is more probably drawn from sections through parts of an embryo system of the stages shown in my figures 17 and 18. It is practically impossible for anyone to obtain a correct interpretation of the embryogeny in stages beyond the four-celled proembryo without the use of dissections.

Figures 14-21 represent stages in early embryonic development from dissections made in a 15 per cent sugar solution. These figures were drawn to the same scale as figures 3-12 and indicate that through enlargement the proembryo may entirely fill the space previously occupied by the archegonium, even encroaching upon the surrounding tissues. However, this increase in size is shown also in serial sections made by the usual technique. Figure 14 is a proembryo showing four 2-celled embryo initials. Each embryonic unit consists of an embryonic cell and a primary suspensor cell (s). The primary suspensor cells may be recognized because they are enlarging slightly and are becoming vacuolated. Figure 15 shows two views of a similar proembryo; the drawing at the left includes a unit found in a high plane of focus which is omitted from the figure at the right.

Figure 16 shows an early embryo system after two of the suspensors have begun to elongate. One unit remains unelongated, and another one is still single-celled. In figure 17 we have the product of two neighboring archegonia such as those of figure 4, which have given rise to six embryos on elongated suspensors and two unelongated units. One unelongated unit has become 5-celled, another one is still single-celled, and one suspensor is binucleate but bears no embryonic cell at its tip. Figure 18 shows a similar condition, seven of the eight embryos being derived from two neighboring zygotes. Embryo units may become aborted in any stage, and other variations may occur. None of the embryos on elongated suspensors have become 2-celled in figure 16; all are 2-celled in figures 17 and 18. In the proembryo of figure 15 one of the embryonic cells has divided before suspensor elongation, so that one of the units of this figure is 3-celled. This and the 5-celled unelongated unit of figure 17 indicate that there is sometimes some variation in the time of division of the embryonic cell and in the manner of division of unelongated units. However, it was found that the division in the embryonic cell nearly always separates this terminal cell into two equal parts by a vertical wall, which is parallel to the axis of elongation of the primary suspensor.

Figure 19 shows four embryos from the same zygote after the suspensors have elongated considerably. One of the terminal embryos is still 2-celled; all the others are 3- or 4-celled. Figure 20 shows a later stage. Though the suspensors have not elongated much more, the embryos have become many

celled and show lobes corresponding to the position of the individual cells of the 3- or 4-celled stages. Some of these lobes may become separated to give rise to small independent embryos, or they may disintegrate so that in either case a delayed form of unequal fractionation of embryos may be found, but the tendency is for the irregularity in the mass of cells to disappear so that in its entirety it becomes a single embryo on the end of the primary suspensor. As the terminal embryonic structures become multicellular, we find a fringe of embryonal tubes elongating around the primary suspensor as shown in figures 21 and 22, and as the suspensor collapses, these elongated embryonal tubes constitute the multicellular secondary suspensor shown in figure 23. Thus we do not have the succession of intervening embryonal tubes  $(e_1, e_2, \text{etc.})$  between the primary suspensor and the more massive secondary suspensor found in Sequoia gigantea but instead an abrupt transition from the primary suspensor to a massive secondary suspensor composed of numerous embryonal tubes nearly equal in length.

From the figures of dissected embryos it appears that a limited period of apical cell growth ensues. The primary suspensor constitutes the first segment of a hemispherical apical cell; the vertical wall of the terminal cell in figures 17 and 18 marks the second segment of an apical cell now wedge-shaped; figure 19 shows the formation of third and fourth segments in different planes. The lobing and tendency toward dissociation shown in figure 20 are no doubt along lines of division of the early segments, still shown in the older stages. The apical cell becomes difficult to follow, and the period of apical cell growth probably does not persist beyond the stages shown in my figures.

Several older stages were observed, but nothing was found which indicated that after the stage of figure 23 the embryos develop in a manner essentially different from other conifers having the same number of cotyledons. Usually only one embryo survives in the matured seed, and embryonic development is completed in the same season as pollination and fertilization. The matured embryo has two cotyledons.

Cytology.—As reported in my previous paper, S. gigantea has n=11 chromosomes. Lawson gave n=16 for S. sempervirens, while I feel fairly certain that in this species n=22. My studies in both species have thus far been made on vegetative divisions in the female gametophytic tissue, but the chromosomes should also be counted in meiosis. It is not especially difficult to count the chromosomes in a late prophase of a gametophytic cell of S. gigantea, but in the corresponding stage for S. sempervirens the chromosomes are difficult to count because of the large number of long chromosomes. I

zygote borne on primary suspensors with embryos showing strong tendency to break up into smaller embryos. June 11, 1936. ×100.—Fig. 21. Later stage with many-celled embryos that have embryonal tubes beginning to form the secondary suspensor. June 18, 1936. ×100.—Fig. 22. Tip of a similar embryo beginning to form embryonal tubes. June 20. ×100.—Fig. 23. Older embryo of many cells on a secondary suspensor composed of many well elongated embryonal tubes. June 20. ×100.

may, therefore, report, provisionally only, that the chromosome number is 22 in the gametophyte of S. sempervirens. The individual chromosomes appear to be of similar width and length in both species, so that the Redwood does not have its larger number as a result of halving of the chromosomes of the other species. The cytology, therefore, indicates a case of polyploidy, which is a phenomenon of rare occurrence among conifers. That the Redwood is a polyploid is indicated also by the relative sizes of the cells and nuclei, the relative size of the body cells for example, the relative width of the pollen tubes, the size of the cells of the proembryo, the width of the primary suspensor cells, and the size of the embryonic cells, wherever my figures of the two Sequoias may be compared.

From this we may infer that of the two species of Sequoia, the Big tree is the more primitive species, and S. sempervirens is the derived or more specialized one. It is not to be assumed that the Redwood was derived directly from the Big tree, since a dozen or more species of fossil Sequoias are known. However, it may be assumed that many of the morphological peculiarities of S. sempervirens which distinguish it from the Big tree, including the unique features distinguishing it from all other conifers, are associated with the mutation to the polyploid condition.

COMPARISON IN EMBRYOGENY OF SEQUOIAS.—The features of the embryogeny in which these Sequoias differ are surprising. The Big tree has a free-nuclear proembryo which does not differ greatly from that of the Taxodiaceae or of other conifers among the Cupressaceae. S. sempervirens has wall-formation at the first mitosis, thus differing from all other conifers. This is evidently a derived condition representing a form of specialization. S. gigantea has a structure which we may regard as a prosuspensor; in the Redwood this feature is omitted. Even if we should regard the elongating cells of the latter which I labeled s as representing instead parts of a prosuspensor, we still find a difference in that there would then be no primary suspensor. I have called these primary suspensors because they may be traced back to the division of one of the cells organized in the proembryo rather than to one of these cells with the division omitted. Only four cells are found in the proembryo of S. sempervirens, and it would be difficult to construct a prosuspensor from a 4-celled proembryo. If the 8-celled stage were used in trying to interpret a prosuspensor for this species, there would be the difficulty of not having these suspensor initial cells arranged properly in tiers, especially in such cases as that of figure 12. The first elongating cells of S. sempervirens, therefore, represent structures that are homologous with primary suspensors of S. gigantea and other conifers.

Rosette cells and rosette embryos are usually present in S. gigantea; they are not generally present in S. sempervirens. If the undivided cells shown in figures 16 and 17 were interpreted as rosette cells and the short unelongated units as rosette embryos,

there would be a difficulty arising from the fact that these may be situated in the same tier with the elongating cells. Provisionally I shall regard them as abortive initials or embryos rather than designating them as rosette elements. They all have a fair chance to form embryos, while rosette cells are usually situated above some kind of suspensor tier which prevents them from having a fair chance to contribute the embryo that may survive. Rosette cells are found in Athrotaxis, according to Saxton and Doyle (1929); so we may have here a closer resemblance between two separate genera (generally accepted as such) than between the two Sequoias.

The interpretation given to the homology of the primary suspensors permits one to recognize a similarity between these elongated cells with respect to the position of the first wall appearing in the terminal embryonic cell. Although this wall appears sooner after elongation in the Redwood than in the Big tree, it nearly always divides the terminal cell into two symmetrical halves. There is an additional similarity in respect to the fractionation of the embryos which takes place some time after the primary suspensors have elongated. This "budding" does not appear to be so clearly marked in the Redwood as in the Big tree. However, there is a difference again in that the embryonal tubes  $e_1$ ,  $e_2$ , etc., elongate at first singly, or at least more or less successively in the Big tree, while in S. sempervirens they seem to elongate more nearly in unison to form a fringe around the end of the primary suspensor. In general the elongating suspensor cells of all kinds become much longer in the Big tree, both relatively and actually.

Though not much could be found concerning variations in the manner of origin of the cotyledons, it is definitely certain that the late embryos differ; S. gigantea is polycotyledonous, and S. sempervirens is dicotyledonous.

As indicated in my previous paper, if we compare the embryo of the Big tree with that of Sciadopitys (Buchholz, 1931) we find considerable resemblance. Sciadopitys (Lawson, 1910) appears to have at least 8 (or possibly 16) free nuclei formed in the proembryo before the mitotic division in which cell walls appear, a difference which might be expected in an egg which is so very much larger. Then each of these genera has a prosuspensor, although this member is relatively smaller in S. gigantea. In both, prosuspensor elongation is followed by the formation of primary suspensors for the respective groups of embryonic units which are borne on the end of the prosuspensor. Both S. gigantea and Sciadopitys may or may not have rosette cells. When the smaller embryonic units on the end of the prosuspensor elongate, the terminal cell divides very promptly and in the same manner in both genera.

We may place Sciadopitys at one end of the series, S. gigantea next to it, possibly Athrotaxis next to this, and S. sempervirens at the end of the series. The smaller and more ephemeral prosuspensor of S. gigantea may be regarded as a prophecy of the

final elimination of this member as we approach greater specialization.

In several earlier papers I took the position that extensive cleavage polyembryony is a very primitive condition among conifers, and that all or nearly all conifers show a compound embryo indicating that they have passed through cleavage polyembryony in their evolution, so that a reduced form of cleavage polyembryony, or eventually the condition of simple polyembryony, are derived conditions. This view, which was developed as a working hypothesis about twenty years ago, has found more and more support in subsequent investigations, and has come to merit serious consideration. These Sequoias lend additional support to this hypothesis, for many other criteria indicate that the Big tree with its extensive cleavage polyembryony (nearly a dozen embryos per zygote) is the more primitive species, and the Redwood with a reduced form of cleavage polyembryony (four embryos per zygote) is the more specialized. The next step in evolution would be only one embryo per zygote, thus ending the series in simple polyembryony.

This condition of polyembryony as well as the series shown in the proembryo which ranges from 16 nuclei at wall formation (Sciadopitys) to 8 nuclei (S. gigantea) and finally wall formation at the first division of the zygote; the n=11 and n=22 chromosome numbers; the well developed prosuspensor (Sciadopitys), a more ephemeral prosuspensor (S. gigantea), and finally the absence of a prosuspensor; all these series indicate direction of evolution of these embryonic structures.

GENERAL COMPARISON OF THE SEQUOIAS.—The account given by Looby and Doyle (1937) may seem to have stressed the points of resemblance between the two species of Sequoia, and the results of their investigations may appear to minimize the importance of the differences, but their observations were mostly limited to the gametophytes. Taxonomists, at least our American botanists, have generally accepted the Sequoias as belonging to the same genus, on the basis of external anatomy. However, thus far too little has been known concerning the details of their internal morphology that have been considered important criteria in the study of relationships among other genera. Among European investigators opinions have been divided, some following our practice of including both Sequoias in the same genus, others insisting that they represent two genera and calling the Big tree Wellingtonia.

It may be timely, therefore, to list the resemblances and the differences. Among the resemblances we find a similarity in the wood structure, a similarity, except for size, in the cones, in the habit of becoming large, long-lived trees, in the general structure of the seed, in the structure of the ovule, in relative position of the gametophytes, and in fertilization. Both have cleavage polyembryony followed by a form of unequal "budding" sometimes resulting in a further fractionation of the embryo; both show much variability in the arrangement of

cells in their respective types of proembryos. Nearly all these internal resemblances as far as they are known are shared by *Athrotaxis* (Saxton and Doyle, 1929) which is everywhere recognized as a distinct genus.

Differences between the Sequoias, including the well known taxonomic differences which may be verified by comparing the full descriptions of both species in Sargent (1902), Jepson (1910), and other monographs, may be listed as follows: S. gigantea becomes the largest tree known, has a characteristic form with relatively stout branches that turn upward at their ends, while S. sempervirens is more slender, becoming the tallest tree known, and has more slender branches that are characteristically horizontal. In fact, the two species differ in eight or more gross taxonomic differences that concern the aspect and habit, the wood, the bark, the stem. etc.

In the Big tree the buds are naked, in the Redwood scaly. Furthermore, there are six or more taxonomic differences between the leaves of the two species. For instance the former has only awlshaped leaves, while the latter has dimorphic foliage, some of its leaves being small and awl-shaped, others much larger, petioled, scythe shaped, and complanately 2-ranked.

The Big tree has no vegetative reproduction, while the Redwood has abundant vegetative reproduction. The former is pollinated in April-May, while the latter is pollinated in January-February, and there are at least seven well marked taxonomic differences between the pollen cones and the ovulate cones of the two species at the time of pollination. Also, S. gigantea has seed cones 2-3 inches long and evergreen, remaining on the trees for many years after the morphological maturity of the seeds, while in S. sempervirens the cones do not exceed 11/4 inches in length and they become dry and shed their seeds within a year. Five or more taxonomic differences are found in the scales of the seed cones, and these differences include a feature not previously pointed out-namely, that the ovules and seeds are borne in two rows on the cone scale of the Big tree and in only a single row in the Redwood. Likewise the embryos of the seeds of the Big tree develop over a 2-year period; those of the Redwood require only a single season for their development. There are also two or three taxonomic differences in the

While most of the contrasts referred to above are only specific differences, they are nearly all distinct enough to be used in a key separating the two Sequoias. However, the difference in the buds, the difference in the history of development and in the persistence of the seed cones, and also the difference in the time required for the maturity of the seeds should be regarded as generic distinctions.

The difference in the size and shape of the pollen tubes is one of the gametophytic differences between the two Sequoias. There is also a difference in the size of the pollen grains, the Redwood having the larger spores. The manner of development of the female gametophytes (as well as their size and shape) is very different in the two species, the Big tree following the usual type of development in conifers, while that of the Redwood is unique. There is also the remarkable feature of a competition between the female gametophytes during development, there are many more archegonia, and the conspicuous irregularly scattered jacket cells, all characteristic of the Redwood. In S. gigantea the megaspore membrane is  $2-3\frac{1}{2}$  microns thick, while in S. sempervirens it is less than 1 micron thick. The number of chromosomes in the female gametophyte differs: n=11 in the former; n=22 in the latter. Among these gametophytic differences more than half are generic distinctions.

In summarizing the embryogeny, nine outstanding differences may be found between S. gigantea and S. sempervirens. The free-nuclear stage in the proembryo of the former, its absence in the latter; the organization of the proembryo into tiers vs. proembryo not organized in tiers; the presence of a prosuspensor in the former and its absence in the latter; are all distinctions of a grade that separate genera and families among other conifers. The difference found in the presence or absence of rosette cells, the difference in the number of embryos derived from each zygote, the difference in the successive versus the simultaneous elongation of embryonal tubes in the formation of secondary suspensors, and the difference in the number of cotyledons, are also distinctions that are of generic rank. In the Big tree the cell volume average of comparable embryonic cells is much smaller than in the Redwood, and the suspensor segments of the former are much more slender, though longer in the former than in the latter, representing differences that are probably only specific in rank.

At least 55 important differences between the Sequoias are known. More than 30 of them are concerned with the external features included in the taxonomic descriptions which have stood for many years. There are also a number of taxonomic distinctions pointed out more recently which should be added to this group, giving a total of three dozen well marked external contrasts between the two Sequoias. A great majority of the gametophytic and embryological differences are characters which are found to be of generic value among other conifers. Almost a third of the total are generic differences. The results of morphological investigations all point to one conclusion, that the two Sequoias belong to different genera.

### SUMMARY

The gametophytes of Sequoia sempervirens were investigated in stages earlier than fertilization. The pollen tubes are relatively wider and not so long as in S. gigantea and contain larger nuclei. They were found in the same position on the sides of the female gametophytes. Several female gametophytes are in

competition within the same ovule, but usually only one of them survives to maturity. Mitotic figures reveal 22 chromosomes in the tissue of the female gametophyte. The megaspore membrane is very thin. The laterally placed archegonia are numerous and occur in groups which are usually adjacent to pollen tubes. These groups superficially resemble the archegonial complex of Cupressaceae, but are surrounded only by irregularly scattered jacket cells rather than a continuous jacket. There is great doubt as to whether the ventral canal nucleus is formed. The division of the body cell, the formation of two male gametes, and their entrance into the eggs were observed. Fertilized eggs are usually found associated in pairs.

When the zygote divides, a wall, which is perpendicular, or nearly so, to the long axis of the archegonium, is formed at the first mitosis. The two resulting cells divide in various planes, so that a number of variations are found in the arrangement of cells in the proembryo. Fifty 4-celled proembryos were observed and the variations in cell arrangements tabulated. Each cell of the 4-celled proembryo may be considered an embryo initial and divides to form a primary suspensor cell and an embryonic cell. As the primary suspensors elongate independently, the four embryo units are separated, while each terminal embryonic cell is divided by walls parallel to the axis of the elongating suspensor. A brief period of apical cell growth may be recognized. No prosuspensor is formed, nor is there a rosette group as found in the other species of Sequoia. Entire embryo initials, suspensor cells, or individual embryonic cells may become aborted in any stage, thus reducing the number of embryos that appear to be derived from one or a pair of zygotes. The primary suspensor is followed abruptly by a massive secondary suspensor composed of numerous embryonal tubes, whereas in the other Sequoia there is a succession of individual embryonal tubes and a gradual transition to a massive secondary suspensor. A further budding or secondary cleavage of the embryo is also possible, but in general the entire embryonic mass borne on a primary suspensor usually contributes a single embryo in which the lobes and irregularities tend to disappear. The single embryo which finally survives embryonic competition forms two cotyledons.

The known features of the gametophytes and embryogeny are compared with those of S. gigantea, Sciadopitys, Athrotaxis, and other conifers. Many differences which are of generic value in other conifers are found between the two Sequoias. There are also many external taxonomic distinctions between them, and nearly a third of all the known differences are of generic value. From these facts it is concluded that the two Sequoias belong to different genera.

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### THE USE OF INDICATOR PLANTS IN LOCATING SELENIFEROUS AREAS IN WESTERN UNITED STATES. I. GENERAL <sup>1</sup>

O. A. Beath, C. S. Gilbert, and H. F. Eppson

ONE of the most important points brought out in connection with the selenium problem of the West has been the discovery (1, 4, 5) that certain native selenium bearing plants may be used as indicators in locating seleniferous soils. These native plants may be relied upon to contain significant amounts of selenium during all or a major part of their annual growth, and hence their presence indicates the occurrence of selenium in the soils upon which they grow. We have consequently attached the name "indicator plants" to such species. An observation of equal importance, in the opinion of the authors, is the influence of many of the highly seleniferous plants in making selenium available to associated vegetation. The significance of this relationship has not been fully appreciated. It is especially important when it results in the absorption of toxic quantities of selenium by grasses, cereal grains, and other farm crops. A later report will discuss recent researches along this line of a practical nature by the authors.

The present paper deals with the different species of indicator plants so far known, their capacity to accumulate selenium, their taxonomic relationships, and their distribution—particularly with reference to the geological formations on which they occur.

It is now known that one or more native seleniferous indicator plants occur in parts of sixteen western states—including Arizona, Colorado, California, Idaho, Kansas, Montana, Nebraska, North Dakota, New Mexico, Nevada, Oregon, Oklahoma, South Dakota, Texas, Utah, and Wyoming. We do

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not propose to review the situation in all these states, nor are we concerned at this time with the question of how completely the data cover the conditions in any one state.

In 1934 (1) we reported that certain native plants of Wyoming could be relied upon to contain selenium if rooted in seleniferous soils derived from rocks of Cretaceous or Tertiary age.

Since then additional geological formations have been found to be capable of supporting seleniferous indicator plants. Data on these are available in published reports (3, 5). In every case, however, the seleniferous areas discovered by the authors were located by means of the selenium indicator plants. One of the most unique correlations of this type was described in a previous issue of this Journal (5) under the heading, "Selenium in Soils and Vegetation Associated with Rocks of Permian and Triassic Age."

Geological relationships.—Since 1934 geobotanical correlation studies have been extended into several of the Rocky Mountain states. One would expect that a seleniferous geological formation in one area or state could be depended upon to also show a seleniferous composition in other areas. It is not surprising, therefore, to find that geological formations such as the Steele, Niobrara, Benton shale, etc., or their equivalents are capable of supporting certain forms of seleniferous vegetation (3, 8, 12) in other states than Wyoming. Reference to geological maps of the United States enables one to locate these areas, at least approximately. The source rocks from which the Tertiary and Cretaceous sedi-

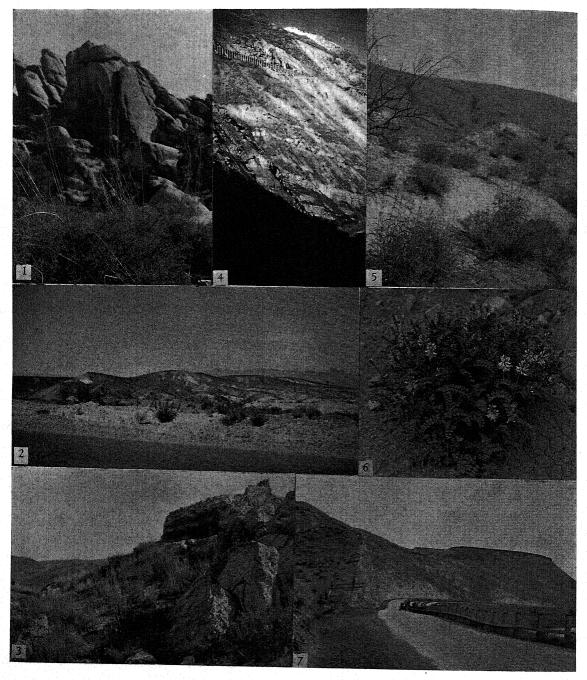


Fig. 1-7.—Fig. 1. Old stems of Stanleya pinnata (Pursh.) Britt. shown to be protruding from a mesquite bush (foreground). White Tank Monzonite rocks in background. Twenty-nine Palms, San Bernadino County, southern California.—Fig. 2, A Stanleya pinnata (Pursh.) Britt. plant in bloom in center foreground. The limestone rock here is probably of Pennsylvanian Age. Near Crystal, southeastern Nevada.—Fig. 3. Massive limestones probably of Palezoic age, near Winsper, Idaho. Indicator plants growing on or near these rocks in this area are very seleniferous.—Fig. 4. A portion of a very seleniferous shale exposed in Provo Canyon, Utah. Rocks are of Paleozoic age.—Fig. 5. A few clumps of Stanleya pinnata (Pursh.) Britt. pre-bloom stage in Tonto Basin Valley, central Arizona. These plants are rooted in sediments which are believed to be Pliocene in age.—Fig. 6. Astragalus sabulosus Jones. A seleniferous indicator plant rooted in sediments of Tertiary or Quaternary age. Upper Verde Valley, north-central Arizona. (Courtesy Joseph Arnold, University of Arizona.)—Fig. 7. The seleniferous bed exposed at the left of the highway extends to the lava cap rock. These sediments are part of the Payette formation which is Miocene in age. Near Glenns Ferry, southwestern Idaho.

ments were derived have been discussed (2, 3, 11) in earlier reports.

In most cases seleniferous rocks carry one or more of the naturally occurring selenium bearing plants. Byers (8), in his original survey (1935), failed to record any significant seleniferous vegetation in New Mexico, Utah, and Arizona. Since that time, however, seleniferous indicator plants have been found in abundance in the areas investigated in these states.

In several type areas in Wyoming it has been observed that above 8500 feet an ecological factor intervenes, so that the principal seleniferous plants disappear, while the seleniferous soil horizons may continue to considerably higher altitudes.

The significance of the selenium indicator plants is particularly important in locating seleniferous areas not heretofore recognized in the West. This paper deals in part with situations of this character. We have selected for detailed study certain areas where one or more of the selenium indicator plants occur. The areas include the White Tank Monzonite (fig. 1) intruded into the pre-Cambrian rocks of the Twenty-nine Palms area in southern California; the limestones of Pennsylvanian age in southeastern Nevada (fig. 2) and central Idaho (fig. 3); the caliche-like deposits in southern Arizona and California; the carbonaceous and limy shales of late Mississippian or early Pennsylvanian age (fig. 4) in Provo Canyon, Wasatch Mountains, Utah; the Tertiary sediments of Pliocene age (fig. 5) in the Tonto Basin; the sediments of Tertiary or Quaternary age in the Upper Verde Valley (fig. 6) of north central Arizona; and the Payette Lake sediments of Miocene age in southwestern Idaho (fig. 7). The seleniferous formations just cited are believed to be new assignments. In none of the above cases were our results negative for the vegetation of any one formation as a whole. The statement made by the authors in earlier publications that some of the indicator Astragali were capable of growing without absorbing selenium is to be interpreted as relative rather than absolute. Plants showing very low amounts are insignificant as livestock hazards. The significance of small amounts of selenium as a physiological factor for the plants was not considered at that time. In most cases, however, where low selenium values are found in native indicator plants, it may be expected that the associated soils will be lean in selenium.

Knight (3, 11) has referred to the source from which much of the Cretaceous seleniferous sediments of Wyoming were derived. The discovery of pre-Cretaceous seleniferous rocks in the area from which the Cretaceous sediments were derived is of interest.

Collections of various Stanleyas have been made in Nevada. The age and character of the rocks supporting much of this vegetation is not known to us. Seleniferous vegetation has also been collected from southeastern Idaho and from Boxelder County, northwestern Utah. Here again the formations are unknown.

The geological formations involved in the study of seleniferous soils and vegetation in western United States include rock types associated principally with three eras (fig. 8)—the Paleozoic, Mesozoic, and Cenozoic.

In order to acquaint readers who are not familiar with the geological nomenclature used in this paper, the following definitions of geological terms and their usage are quoted from a previous bulletin (11) on this subject:

The geological column comprises all the rocks of the earth's crust. It is divided into time terms and rock terms. The divisions of the geological time scale and corresponding rock terms are:

ime tern	1		Rock tern
Era		100	
Period		 	System
Epoch		 	Series
Age		 	Group
Stage		 	Formation

Era. The largest division of geological time.

Periods and systems. Geological eras are composed of a number of periods (time). Each period is represented by a system (rocks)....

Formation. The formation is the fundamental unit in the local classification of rocks. A formation may be a few feet thick or it may be five thousand feet thick. A formation may vary greatly in thickness and character from place to place, and a single formation may be composed of an alternating succession of various rock types, such as sandstones, shales, etc. Formations are names from geographical localities. For example, the Sundance formation is named from the town of Sundance, Crook County, Wyoming. . . .

Member, lentil, tongue, and lens. These are subdivisions of a formation. Specially developed parts of a varied formation are called members if they are of considerable geological extent; lentils if they are of slight geographic extent; tongues if it is known that in one direction they wedge out, laterally, between sediments of different character; lenses if they wedge out laterally in all directions.

Bed, stratum, and layer. These are the smallest units recognized in classification.

Broadly interpreted, the geological column of central Wyoming is representative of the formations or their equivalents in the Rocky Mountain region. In the discussion of geobotanical data in this paper, the authors refer from time to time to the Wyoming generalized geological column (fig. 8) in order to establish the continuity of the seleniferous character of rocks of equivalent age.

In the Paleozoic era the oldest seleniferous rocks so far discovered occur in late Mississippian or early Pennsylvanian. A representative formation is well exposed in Provo Canyon, Wasatch Mountains, Utah.

From late Paleozoic through the Mesozoic and Cenozoic the occurrence of selenium is variable. Many formations in the Permian, Triassic, and Jurassic systems do not contain enough selenium to produce toxic vegetation. Beginning with the Morrison formation (late Jurassic) and continuing on through the Cretaceous system, selenium occurrence

### GENERALIZED GEOLOGIC COLUMN FOR CENTRAL WYOMING

ERA	SYSTEM	SERIES		GROUP		FORMATION	REMARKS	
		Pliocene				North Park		
		Miocene				Harrison		
				White River		Brule		
		Oligocene				Chadron		
•						Uinta	Non-marine	
Cenozoic	Tertiary		M.			Bridger		
		Eocene		W: - 4 D:		Lost Cabin		
			L.	Wind River		Lysite		
						Greybull		
		Paleocene				Fort Union		
						Lance	Brackish-water	
				Montana		Lewis	Marine and	
***						Mesaverde	Non-marine	
						Steele		
						Niobrara	Marine	
		Upper				Carlile		
	Cretaceous			Colorado	c	Frontier	Marine and Non-marine	
					enton	Mowry	Marine	
Mesozoic					В	Thermopolis		
MESOZOIC						Upper Cloverly	Largely Non-marine	
		Lower P			Dakota	Middle Cloverly		
					Dal	Lower Cloverly	Montmarine	
	Jurassic	Upper				Morrison	Non-marine	
	041 45316	Obbet				Sundance	Marine	
						Jelm	Non-marine	
	Triassic					Chugwater	Largely	
						Dinwoody	Non-marine	
	Permian	Middle			77	Phosphoria	Marine	
Paleozoic	Pennsylvania	10.00				Tensleep	Marine and Non-marine	
	Mississippian					Madison	Marine	
	Cambrian					Deadwood	Marine	
pre-Cambrian		-					Igneous and Metamorphic ro	

Fig. 8. The geological column of central Wyoming. This column is used as a standard for comparative purposes in this paper.

is common and practically continuous. Selenium in Tertiary sediments is more or less localized. This feature was discussed in earlier publications (3, 5, 11). Seleniferous rocks have been found in formations of Paleocene, Eocene, Oligocene, Miocene, and Pliocene age.

In the Hanna Basin, of Carbon County, Wyoming, the late Cretaceous and Eocene rocks are very thick. Here the Medicine Bow formation is overlain by several thousand feet of conglomerates, light and dark colored shales, brown sandstones, and coal beds. This complex aggregate of varied rock types has been divided into the Ferris and Hanna formations. Much, if not all, of the rock debris which make up these formations was derived locally from the adjacent mountains. The Ferris formation, from its basal contact with the Medicine Bow to the top, represents an exposure of considerable magnitude. The beds stand at high angles.

Xylorrhiza parryi (woody aster) in full bloom was collected on the same day from raw shale outcrops of the Ferris formation along a diagonal traverse of four miles. Woody aster is dependable as an indicator of seleniferous soil; and the selenium content of this plant provides a fairly accurate index of the corresponding selenium content of the soil. The areas from which the aster was taken are designated as A, B, C, D, and E (fig. 9). It will be observed

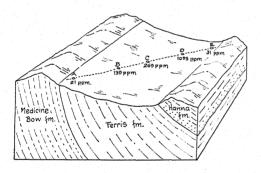


Fig. 9. Diagram showing the variation in parts per million in the selenium content in specimens of *Xylor-rhizu* collected on a diagonal traverse of the Ferris formation, Carbon County, Wyoming.

that at the point marked D the plants reached their maximum selenium content; at point A, their lowest. Soil selenium in the highly seleniferous area was found to be 4.0 parts per million, while at location A it was only 0.5 part per million. These data illustrate selenium localization in Tertiary rocks. However, in certain areas these rocks may be uniformly seleniferous when derived from source material of uniform selenium content.

SELENIUM INDICATOR PLANTS.—The primary selenium indicator plants as defined by the authors are those universally seleniferous in their annual cycle of growth. The primary group of indicator plants is centered chiefly around four genera. These include species of Stanleya, Oonopsis, Xylorrhiza, and Astragalus. The authors included Mentzelia deca-

petala in the original list of indicators. Because most species in the genus Mentzelia do not absorb selenium in toxic quantities and those that do are irregular in distribution, this genus is now placed in the secondary or intermediate class. In most instances the maximum concentration of selenium occurs in the plant's early growth. In soils with low selenium values the selenium content of the indicator plants will usually be correspondingly low.

Although some samples of the primary indicator plants have given negative tests for selenium, analysis of these species from the same soil areas at a time when the initial growth was starting has invariably resulted in obtaining positive tests for selenium. In one instance the past seeding stage of one of the major indicator plants, Astragalus bisulcatus, showed only 3.5 parts per million selenium, but a composite sample from the same area the following spring showed 135 parts per million selenium. The surface foot of soil (composite) contained 0.49 parts per million selenium.

Moxon (12) found a few Astragalus racemosus plants to be non-seleniferous when partly or fully matured. To determine definitely whether this or any other species of certain seleniferous groups of the Astragali can grow and develop normally without absorbing some selenium, it would be necessary to analyze plants at several stages of growth from the same soil area. Occasionally large thrifty Astragali may be observed approaching maturity without producing fruiting heads, or plants may have their fruits drop prematurely owing to injury by insects. More selenium accummulates in the seeds and pods of the Astragali than in the vegetative parts, consequently occasional samples may be obtained that are not representative of normal conditions.

Trelease (13) has shown that for certain species of Astragalus selenium may be an essential microtrophic element.

Species of Stanleya. - In a discussion of the species of Stanleyas that are absorbers of selenium, it is not possible to report on all of them at this time owing to the fact that the authors have not had an opportunity to obtain sufficient material for chemical analyses. Those species that are known to be dependable in their selenium absorption are: Stanleya pinnata (Pursh) Britt., S. bipinnata Greene, S. integrifolia James, S. glauca Rydb., S. viridiflora Nutt., S. tomentosa Parry. It is generally true that the amount of selenium in these plants is a fairly reliable index of the quantity of selenium in the soils in which they grow. Exceptions occur, as would be anticipated. A Stanleya sample (not identified) collected near Battle Mountain, Nevada, gave a negative test for selenium. The surface soil (1st foot) had 2.20 parts per million selenium. The plants, as received, were past the seeding stage and considerably weathered. Young thrifty plants from the same area would probably be definitely seleniferous in a soil carrying this amount of selenium providing that the seleniferous condition continued to the depth of the feeding roots.



Fig. 10-14.—Fig. 10. The club-like heads of Stanleya tomentosa Parry, Owl Creek Valley, Hot Springs County, Wyoming. The formation here is of Triassic age.—Fig. 11. Aster adscendens Lindl. in common with other species of the genus usually occurs in dense patches. It is seleniferous when rooted in selenium bearing soils.—Fig. 12. Xylorrhiza parryi (A. Gray) Greene. Each clump is a potential source of available selenium. Southern Albany County, Wyoming.—Fig. 13. Stations indicate areas from which comparable plant samples were taken for selenium analysis. Central Albany County, Wyoming—Fig. 14. In the immediate foreground are several clumps of seleniferous Astragalus praelongus Sheld. (Pre-bloom.) These plants are growing in rocks of Triassic age (basal). Near south entrance of Zion National Park, Utah.

The Stanleya species vary in their selenium absorbing properties. Stanleya pinnata and S. bipinnata normally will be more seleniferous than S. viridiflora under similar stages of growth and environmental conditions. Specimens of S. viridiflora of unusual growth and thriftiness have in several instances given very low selenium values. This is particularly true of samples from certain areas in Idaho and Nevada. Their value as indicators of a seleniferous soil, however, is significant in that their occurrence frequently suggests the presence of other, highly seleniferous plants in the immediate vicinity.

Stanleya integrifolia usually carries a toxic amount of selenium but does not run as high as S. pinnata or S. bipinnata under similar conditions of growth and habitat. Stanleya tomentosa Parry has not been reported to occur (fig. 10) outside the Big Horn Basin, north and central Wyoming. There are a number of Stanleya species in the western United States about which no selenium data are available. Representative valid species are S. runcinata Rydb., S. albescens Jones, S. canescens Rydb., S. elata Jones, and possibly others.

Species of Oonopsis.—The species in this genus that have been examined for selenium show a decided affinity for it. The present list includes: Oonopsis condensata A. Nels., O. argillacea A. Nels., O. foliosa (Gray) Greene, (Aplopoppus fremontii Gray), O. engelmannii (Gray) Greene.

Of the several type species analyzed for selenium, Oonopsis argillacea has shown the lowest average

content under comparable conditions.

The species not analyzed are: Oonopsis multicaulis (Nutt.) Greene, O. wardii (Gray) Greene, O. monocephala A. Nels.

The tendency for some species to yield high results and others average to low is quite generally applicable to all the seleniferous indicator plants.

Species of Xylorrhiza.—The most significant species of the Xylorrhizas in so far as selenium absorption is concerned is X. parryi (Gray) Greene. It also has a wider distribution than any other species in the Rocky Mountain area. Other seleniferous species include: X. villosa Nutt., X. venusta (M. E. Jones) Heller, X. glabriuscula Nutt.

Analysis of X. villosa collected from a number of soil types shows this species to be a moderate to low absorber of selenium compared with X. parryi and X. glabriuscula under comparable stages and condi-

tions of growth.

Species that have not been analyzed are: X. coloradensis (Gray) Greene, X. brandegei Rydb., X.

lanceolata Rydb.

The seleniferous *Xylorrhizas* as a group yield the highest amount of selenium in the early stages of growth. After the seeds are well developed, the tops of the plants wither and are scattered by weathering.

Aster abatus Greene, originally named Xylorrhiza tortifolius Gray, has been collected from several areas in California and Utah, but no selenium has been found in any of the samples. (See results on Asters.) By the criterion of selenium absorption, this species is not a Xylorrhiza. From the assembled data, it appears fairly certain that all the dominant species of the genus require selenium for their existence. Since Xylorrhiza tortifolius (Aster abatus) is negative in this respect, we conclude that X. tortifolius is not a Xylorrhiza but an Aster. Type specimens from Utah and California contained no selenium.

Species of Astragalus.—This is a particularly interesting genus and constitutes the most important group of the selenium indicator plants. It is the only genus in which one can attempt to separate by genetic differentiation selenium-absorbing species from non-absorbing species.

For example, no Astragalus having an inflated pod has as yet been found capable of absorbing selenium in toxic quantities from naturally occurring seleniferous soils, whereas all the available evidence points to universal absorption by species characterized by a "bisulcate" pod. Marcus E. Jones (10) classified the species of Astragalus of the United States into twenty-nine groups. His division was

based upon genetic characteristics. As a starting point in our investigations of the seleniferous Astragali of the western states, we chose Jones' classification for correlation studies. Of the twenty-nine groups listed by Jones, we have had an opportunity to examine one or more species in most of them. The dominant selenium absorbing Astragali now known include species in but five of the twenty-nine groups. These are:

Astragalus haydenianus Grav . Astragalus oocalycis Jones Astragalus scobinatulus (Sheld.) Bisulcati Astragalus bisulcatus (Hook.) Gray Astragalus urceolatus Greene Galegiformes Astragalus racemosus Pursh (Astragalus flavus Nutt. OcreatiAstragalus flaviflorus (OK.) Sheld. Astragalus confertiflorus Gray Astragalus toanus Jones Podo-sclerocarpi Astragalus grayi Parry Astragalus pectinatus Dougl. Astragalus preussii Gray Astragalus preussii var. latus Jones Astragalus pattersonii Gray Preussii Astragalus praelongus Sheld. Astragalus sabulosus Jones Astragalus eastwoodae Jones Astragalus limatus Sheld.

It is evident from our preliminary studies that there is a definite tendency toward a genetic segregation of the selenium absorbing properties so characteristic in some Astragali and entirely lacking in others. The Homalobi group, for instance, includes some twenty-three type species widely distributed in western United States, and yet none of the species so far examined have carried significant amounts of selenium. The large group of Inflati, the Reventi-arrecti, Uliginosi, Hypoglottides, etc., also are non-absorbers. The authors have not been able to obtain representative species in the Sparsiflori, Alpini, Strigulosi, Atrati, or Micranthi groups.

In presenting data to support the hypothesis that some Astragali are seleniferous and others not, depending upon genetic relationships, the authors wish to go only as far as the facts would warrant. Until all the important species in the twenty-nine groups as outlined by Jones have been carefully checked, one cannot hope to arrive at final conclusions.

Among systematic botanists, both past and present, we find divergent opinions on Jones' revision of the Astragali. This is a natural consequence particularly in dealing with a genus of such magnitude and complexity. In approaching the study of seleniferous Astragali from our point of view, we have found the revision of the Astragali by Jones to be the most helpful of any of the botanical references consulted. In behalf of Jones' monograph on Astragalus it must be admitted that it was prepared by a botanist thoroughly familiar with field conditions.

In so far as our investigations have gone, it is quite significant that the exceptions to our hypothesis are infrequent. One of the most prominent of these exceptions has to do with Astragalus drummondii. This species is placed in the Galegiformes group along with A. racemosus. Astragalus drummondii normally does not absorb selenium in toxic quantities. On the other hand, A. racemosus is a very dependable absorber of selenium and hence an indicator of seleniferous soil. No explanation of this variation seems to be possible at this time.

The fact that we have been able to predict that certain Astragali should be seleniferous and then confirm the prediction by actual chemical analysis warrants the suggestion that our hypothesis has some economic value. For example, Astragalus toanus Jones belongs to the Podo-sclerocarpi group. In this same division are A. grayi and A. pectinatus, which we had early found to be seleniferous. Samples of A. toanus were therefore obtained from Utah, Nevada, and Idaho. They were found to be seleniferous. In addition, A. toanus is confined exclusively to soils of a seleniferous character. It becomes a primary selenium indicator plant in the sense that it contains selenium and may be expected to occur only on a seleniferous soil.

Associated with the seleniferous Astragalus pattersonii, A. praelongus, and A. sabulosus in the group classified as Preussii is Astragalus preussii. A type sample taken from an area near Las Vegas, Nevada, was found to be definitely seleniferous on a massive limestone that carried only 0.54 parts per million selenium. The genetic association placed A. preussii in the suspected class of selenium absorbing Astragali. For this reason it was sought out from other Astragali in this area. Also, two collections of a variety of A. preussii from Utah and one from New Mexico were seleniferous.

In considering data supporting the correlation of certain seleniferous Astragali species with genetic relationships, it should be borne in mind that our interpretation of a selenium absorbing plant is based upon the dependable capacity of these plants for acquiring selenium from naturally occurring seleniferous soils. To illustrate this point from field evidence, A. carolinianus may be considered. Astragalus carolinianus L. has been collected from several typical seleniferous soils in Wyoming. Analyses of these samples show that it is definitely not a selenium indicator plant in this state. Byers (8) (Third report) has reported this species to be quite generally seleniferous. The fact that it is not a dependable absorber in Wyoming at any stage of growth places the species outside the primary indicator plants. In view of the fact that Byers gave no data from Wyoming on A. carolinianus, we are unable to check the identification of his material. Type species in the group Uliginosi, to which A. carolinianus belongs, are not selenium bearing even on decidedly seleniferous soils.

Much remains to be done in the correlation study of the selenium bearing Astragali into genetic segregations. The authors have been retarded in this investigation through their inability to secure type

samples from areas far removed from Wyoming. Preliminary investigations point toward a definite trend, but whether or not such a generalization is tenable for the Astragali as a whole, with so many key species still not analyzed, remains to be ascertained. The possibility of the genetic relationships entering into the selenium study was not initially considered. It was only after several hundred analyses had been made that our attention was directed to the fact that many of our data offered a kind of correlation not applicable to other seleniferous plant genera.

Brandegee (7) (1876) called attention to the resemblance in odoriferous principle between the flowers of Astragalus haydenianus and those of A. bisulcatus. Jones (9) (1895) mentioned Astragalus pattersonii as being a nauseous poisonous weed. Beath (6) (1917) noted that the water extracts of A. bisulcatus were markedly odoriferous. It is now recognized that many of the seleniferous Astragali emit an odoriferous principle, varying in intensity of course but nevertheless persistent. It is believed that every sample of Astragalus having the characteristic odor has been seleniferous. To the experienced plant collector this odoriferous principle is not only a fairly accurate qualitative indication of selenium, but in addition it can be depended upon for a prediction as to whether an Astragalus specimen is moderately or highly selenized. In 1937, while the senior author was seeking seleniferous vegetation in northeastern Arizona on the basal Triassic geological formation, he found in the vicinity of Winslow an Astragalus that emitted the typical odor so commonly noted in all the Bisulcati group. Later, this plant was identified as Astragalus praelongus. A. sabulosus from the Upper Verde valley in Arizona was also noticeably odoriferous. Both of these Astragali proved to be highly seleniferous.

INTERMEDIATE SELENIUM-BEARING PLANTS.—Researches have brought to the foreground the economic significance of plants of several genera which, though not restricted to selenium-bearing soils, are capable of absorbing selenium in toxic quantities when growing on seleniferous soils. Many species in this group are grazed by livestock. We propose to classify plants of this kind as intermediate or secondary indicators. A few representatives in this class are species of Aster, Atriplex, Grindelia, Machaeranthera, Sideranthus, Mentzelia, Pentstemon, Gutierrezia, etc. (8, 12). This is a very difficult group to evaluate. Preliminary field evidence shows that some native plants can absorb selenium from its naturally occurring nearly insoluble form in rocks and soils and that a host of other native plants cannot do this. In many very toxic areas the soils have become enriched with selenium through decomposition and weathering of the highly seleniferous plants-both past and present. In instances of this sort it is certain that any vegetation growing under this influence will be seleniferous. The evidence is most convincing on this point. Soils have

been taken from representative toxic areas and submitted to both chemical and cropping tests.

Naturally there is an overlapping in the secondary group. One cannot distinguish clearly between those plants that can absorb selenium from its naturally occurring source and those that must depend upon its being made available to them. Chemical analyses have shown that several species of unrelated plant genera are capable of absorbing selenium from naturally occurring seleniferous soils and in amounts to be toxic to livestock. These same species may be perfectly good forages on very lean or selenium-free soils. Unfortunately there is no physical indication as to whether these plants are good or bad. Supplemental field evidence, of course, can be obtained, provided the geological structures that are known to carry seleniferous rocks are recognized. This is a simple matter where formations of importance are exposed, but it is difficult to follow through completely.

Asters.—Representative species of Asters that have been found to be capable of absorbing toxic quantities of selenium from raw shales include Aster commutatis Gray, A. adscendens Lindl. (fig. 11), A. glaucus T. & G., and A. ericoides L. Range survevs have included several collections of seleniferous Asters believed to be different from the species listed above, but identification was not possible because of the lack of flowers or fruits. The list of seleniferous Asters may, therefore, be considered conservative. The Asters are largely perennials. They propagate easily by means of underground rootstalks. From an economic viewpoint they merit special mention because of their wide distribution, dense growth, palatability to livestock, tolerance to frosts, and adaptability to a variety of climatic and soil conditions. Asters frequently occur in hay meadows. If the soil happens to be seleniferous, the toxic Asters are cut along with the hay. In addition to this, the hay also may be toxic if it has absorbed readily available selenium. This influence of Asters is in no way restricted to meadow lands; on some grass ranges the seleniferous Asters occur in dense growth and markedly affect the associated palatable forages.

Machaeranthera.—The principal species considered in this genus is M. ramosa A. Nels. It is a biennial. On a moderately seleniferous soil this species has yielded as high as 1600 parts per million of selenium. The soil from which this particular collection was taken was found to contain 2.08 ppm. in the first foot, 2.56 ppm. in the second foot, and 7.23 ppm. selenium in the third foot. The basal leaves of M. ramosa are succulent and very palatable. Other species have not been investigated.

Sideranthus grindelioides (Nutt.) Rydb. (Aplopappus nuttallii T. & G.).—S. grindelioides has been found to be definitely seleniferous when associated with soils containing naturally occurring selenium. This and related plants are grazed by sheep during the fall and winter months. While not as seleniferous as Machaeranthera ramosa on the same

general soil types, S. grindelioides and other species are to be considered as possible poisonous plants. In addition, their density of growth is such that a considerable amount of available selenium reverts to the soils in which they grow. Favorable moisture conditions in the fall of the year result in the development of new basal leaves that remain green and attractive to grazing livestock.

Grindelias.—The Grindelias as a group are hardy biennials and perennials. For the most part they are unpalatable. Being prolific seed producers, new stands quickly occupy waste places and abandoned fields. Their selenium absorbing properties are indefinitely established. From the analysis of a large number of collections from such soils as are known to be definitely seleniferous, it is believed that the Grindelias do not absorb selenium to any marked extent from raw shales and soils. Our opinion is that some selenium derived from other plants has to be available to them in order to be absorbed in toxic amounts. Probably G. squarrosa has received more attention than any other species in the genus. Because so many samples taken from seleniferous soils have given negative tests for selenium, the authors have concluded that the species of Grindelias so far examined are not dependable in their selenium absorbing properties.

Atriplexes.—Atriplex canescens and A. nuttallii are representative species that may be seleniferous. As in the case of the Grindelias it is not possible to make clear-cut statements that will prevail over a wide set of conditions. A. canescens has been found to be somewhat more dependable as a selenium absorbing plant than A. nuttallii under the same soil environments. In either case a number of inconsistencies have been observed. It seems quite certain that to absorb enough of the mineral to be significant, A. nuttallii requires a soil that carries some easily available selenium. A. canescens, however, has been found to be a dependable absorber on soils of Pierre and Niobrara origin. From other types of soils the data are too variable to permit definite conclusions at this time. Atriplex pabularis, A. argentea, and A. confertifolia (shadscale) normally have not been found to be seleniferous.

Grayias.—Grayia spinosa collections from a number of western states have been found to be very lean in selenium. Grayia brandegei collected from an area near Rifle, Colorado, was rather highly seleniferous. Isolated cases of this kind are not dependable, because of the possibility of the plants having been influenced by associated seleniferous vegetation. Further collections will be required to determine the status of the species in this genus.

Mentzelias.—Species in the genus Mentzelia that may be expected to absorb selenium in toxic amounts are exceptional. M. decapetala is likely to be associated with a seleniferous soil. On the other hand, it is not usually high in selenium even on soils highly seleniferous. A large number of species collected from various areas in the western states shows that

the group as a whole is of little importance in so far as selenium absorption is concerned.

Pentstemons.—No attempt has been made to investigate the many species in this genus for their selenium absorbing capacities. P. exilifolius A. Nels. frequently occurs in association with the seleniferous Xylorrhizas, Stanleyas, Oonopsis, etc. The amount absorbed is small at any stage of its growth. The tender basal leaves are palatable to range livestock. Under favorable moisture conditions in the fall P. exilifolius may appear as a regrowth and prove attractive to grazing animals. This species has been collected from a number of areas in Wyoming.

Converter plants.—In Wyoming it is generally recognized that localized areas do occur in which practically every type of plant indigenous to the region is seleniferous. Consequently it is difficult to evaluate adequately the average run of selenium bearing plants when complicating influences are involved. The naturally occurring seleniferous soils of the West, considered broadly, are incapable of supporting seleniferous grasses, farm crops, cereals,

and vegetables unless the selenium is in an available form derived from various native plants. Highly seleniferous plants, both of the primary and intermediate types, are continually contributing to a selenium enrichment in those areas where their density of growth is appreciable. Furthermore it is believed that all seleniferous vegetation contains selenium in an organic form.

Soil selenium, when taken up by plants, becomes orientated into one or more organic complexes. The vegetative phase, then, is characterized as one exerting a positive force in converting soil selenium into an organic form. This is a unified action because all plants termed converters are believed to be producing organically combined selenium. In the aggregate, the millions of tons of seleniferous vegetation annually developed in the western United States mean that a very considerable amount of soil selenium has become organically constituted. For example, an average growth of Xylorrhiza parryi (fig. 12) occurring on a moderately seleniferous soil will yield approximately a pound of selenium

Table 1.\* Stratigraphic section of shale at Canyon Glen Camp Grounds, Provo Canyon, Utah.

Bed N		Thickness in feet	Selenium ppm.
20	Shale, chocolate-brown color at base, grades to dark black thinne		
	bedded shale		3.6
19	Limestone, forms massive cliff above water flume; is dark black	to	
	blue-gray in color, some sandy limestone present		1.5
18	Shale, very black, has seams of yellowish-brown ferruginous materia		
	at very top and at various places in bed		34.3
17	Limestone, very shaly, blue to black with brown and yellowish-brow	/n	
	seams		0.6
16	Shale, light-brown to dark chocolate-brown, gypsiferous in seam	ıs.	
	Forms first shale outcrop near flume		25.1
15	Limestone, blue to gray, some shale which is brownish in color; t		
	bed grades from true limestone at base to sandy and shaly limeston		
	at top	25	12.0
14	Shale, variegated, mainly black with some chocolate-brown colors	24	96.3
13	Limestone, very shaly, dark blue to black	29	2.6
12	Shale, very dark black, thin-bedded	20	7.0
11	Limestone, gray to black in color; hard	71/2	0.5
10	Limestone, very sandy, weathers light-brown	21/2	0.5
9	Shale, limy in part, dark black to gray	20	0.6
8	Sandstone, reddish-brown to buff, coarse grains or grit up to 4 mi	n.	
	in diameter		
7	Limestone, shaly, dark black to dark brown, contains numerous sma	ıll	
	Lingulids	. 11	1.0
6	Limestone, blue to dark black, hard, weathers blue-gray	14	1.0
5	Shale, chocolate-brown, bedding 1 in. to 4 in.; shales are lighter col- towards the top, and various fresh- or brackish-water Pelecypo	or Je	
	present	12	7.5
4	Shale, dark black, carbonaceous in part, and ferruginous in part, be		1,5
	ding less than 1 inch	3	1.8
3	Limestone, dark blue, in part shaly	41/2	
2	Sandstone, reddish-brown, very gritty, with coarse fragments up to	* <del>**</del> /2	• •
	mm. in diameter	4	1.4
1	Limestone, blue, which extends to bottom of railroad cut, or to ra	· · · *	1.4
	road tracks	. 7	0.4
	Total thickness		U,-#

a Permission to use the lithological data in this table was given by U. S. Geological Survey.

per acre. A 2,000 acre patch would thus absorb a ton of selenium. All of it would be converted to the organic form in the *Xylorrhiza*.

ORIGIN OF SELENIUM IN SOILS.—Selenium occurrence (3, 5, 8, 11, 12) in rocks has been described in earlier publications. The general conclusion was advanced in these publications that vulcanism had exerted a marked influence. Although the authors were not satisfied with attributing the origin of many seleniferous areas entirely to vulcanism, yet not enough evidence was at hand to clarify questionable cases. As our investigations were continued, it became evident that seleniferous soils occurred in a number of states where vulcanism, if present at all, must be considered in a broad manner. Rocks of late Mississippian or early Pennsylvanian age (Carboniferous) have been studied in several areas. A particular shale formation in Provo Canyon, Wasatch Mountains, Utah, is one case in point. The carbonaceous beds in this formation are very seleniferous (table 1). This formation is among the most highly seleniferous geological formations in the western United States. Although the extent to which it supports seleniferous plants has not been determined, a sample of Aster glaucus taken from one of the beds was found to be seleniferous.

An inspection of the data in table 1 shows that the selenium is concentrated primarily in the carbonaceous shale. The selenium in the limestone rock is low. The carbonate content, was found to vary roughly in inverse proportion to the selenium present. Consequently the rocks having the most carbonate contained the least selenium. This relationship suggests that the selenium concentration in this formation is more closely related to the carbon cycle (plant) than to the carbonate.

There is no evidence that volcanoes were active in the region during the time this formation was laid down. The question arises as to the source of the selenium in the beds of this formation: Did it come from igneous rocks by erosion and transport of seleniferous materials? Was there absorption of selenium from the brackish waters by marine organisms and subsequent accumulation on the floor of the sea? Did plants of high selenium-absorbing properties growing in shallow basins and on flood plains become incorporated in the sediments? These are a few of the possibilities that suggest themselves.

The limestones of Pennsylvanian age in southeastern Nevada, the localized caliche-like deposits in southern Arizona and California and the limestones (Carboniferous) in central Idaho are other examples of rocks that not only show a seleniferous composition but are capable of supporting selenium bearing vegetation. Other influences than vulcanism may have contributed to the selenium enrichment in rocks during past geological periods.

Several kinds of selenium enrichment are in evidence at the present time. For the most part our data have been obtained from native vegetation rooted in soils. Very little information has been ad-

vanced concerning plants growing in streams, lakes, and seas. An aquatic plant (water cress) rooted into the water of Warm Springs Creek, Lemhi County, Idaho, showed upon a chemical analysis 16 parts per million selenium. The selenium content of the water was so meager that a chemical test failed to indicate its presence. When viewed as operating over a period of years, it is clear that aggregate plant developments of this kind would gradually find their way to adjacent soils or would become a part of the sediments in stream channels and drainage basins.

Seleniferous rocks, regardless of the geological formation with which they are identified, hold the selenium in a highly insoluble form. In addition to the laboratory evidence on this point it is known that the number of native plant species that can absorb selenium from the raw rock and shales is small compared with the total forage cover indigenous to any seleniferous area.

QUANTITATIVE SELENIUM ANALYSES.—Exploratory studies of seleniferous vegetation in the western states have brought out several significant points in regard to the interpretation of selenium analyses. In many instances a quantitative result may be correctly obtained and reported, but as representing the actual selenium content of a particular plant in situ or over any considerable area it may have merely qualitative significance, and then only for a particular stage of growth, which may or may not be the phase with the maximum amount of selenium.

Within a comparatively small area (table 2) there may be variations of such magnitude in the selenium content of the same species of plants that it would be hopelessly confusing to give a single value as representing the actual selenium relationships of the area as a whole. A review of the data from a detailed study of a typical seleniferous shale formation shows how easily one can be misled in results if sampling is confined to a few samples taken at random. The area chosen for critical study was on a Niobrara outcrop with associated talus and wash. Three stations were established. Station 1 (fig. 13) was in an area designated as wash about one-half mile from the main outcrop. Station 2 was an intermediate station. Station 3 was on the raw shale proper or escarpment. Composite collections of

Table 2. Variations in selenium content of plants growing within a small area.

ation 1	Station 2	Station 3
23	44	47
330	1614	2590
a	1642	860
300	253	135
	67	161
664	883	3250
1190	244	1252
	330 a 300  664	330 1614 a. 1642 300 253 . 67 664 883

<sup>&</sup>lt;sup>a</sup> Blank spaces indicate that representative material was not available.

plants indigenous to the general area were made on the same day in September from all three stations. The samples were analyzed while still fresh. The results are reported as parts per million of selenium on a bone dry basis.

Soil samples were taken at certain depths at stations one and three. The results given in table 3 show the selenium content of the soils at each foot level to a depth of four feet.

Table 3. Soil analyses.

Depth		Station 1	Station 3			
0"-12"	8.1	ppm. Selenium	20.4 ppm. Selenium			
12"-24"	6.8	ppm. Selenium	15.8 ppm. Selenium			
24"-36"	2.9	ppm. Selenium	11.7 ppm. Selenium			
36"-48"	7.7	ppm. Selenium	8.4 ppm. Selenium			
48"-60"			7.8 ppm. Selenium			

These soil analyses show very clearly that a highly seleniferous condition exists in this particular area. Furthermore the surface samples from station 3 are shown to be from two to three times as seleniferous as those from station 1. Yet the data on the selenium in the plants do not suggest this marked difference. These results also show how misleading it is to take a sample of the surface soil and expect that it will represent the selenium content of the lower soil in which the plants are rooted.

If an analysis were to be made of the same plant species as given in table 2 at some other stage of growth, particularly in the initial phases, one would find a greatly increased amount of selenium in all of them with the exception possibly of *Oonopsis*. Whether or not the same distributional relationships would prevail at the three locations could not be conjectured.

When seleniferous native range plants are permitted to dry out before a selenium analysis is made, the loss of selenium will be appreciable in some cases and negligible in others. Fresh samples of Astragalus bisulcatus, A. scobinatulus, A. haydenianus, and

A. racemosus have been found to lose more selenium upon drying out than A. pectinatus at the same general stage of growth. Loss of selenium through drying has not been found to be proportional to the concentration of selenium in Astragalia. Astragalius racemosus and A. bisulcatus were found upon drying to lose about the same amount, whether moderately or highly seleniferous.

### SUMMARY

This paper treats the geographical and geological occurrences of 32 species of native selenium indicator plants, one or more of which are known to occur in twelve western states. Native selenium indicator plants are defined as those which contain significant amounts of selenium during all or a major part of their annual growth and thrive only in rocks or soil which contain selenium. The paper also treats a number of intermediate selenium-bearing plants. These intermediate plants may contain selenium when rooted in selenium-bearing rocks and soils. They are not restricted, however, to such occurrences. The data presented set forth the following: selenium is not a constituent of all rocks; the amount of selenium absorbed by a given species varies greatly at different horizons within a given formation; the areas containing seleniferous plants in which the selenium is present in toxic amounts are limited; a very significant percentage of the vegetation growing on seleniferous soils does not absorb selenium in toxic amounts; seldom are indicator plants formed above an elevation of 8500 feet; all species of Stanleya, Oonopsis, and Xylorrhiza thus far studied are classed as indicator plants; only those species of Astragalus which occur in five of the 29 groups as classified by Jones are seleniferous; the characteristic odor given off by some seleniferous Astragali is evidence that selenium is present. The origin, form, and distribution of selenium in soils is considered.

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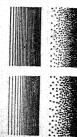
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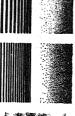
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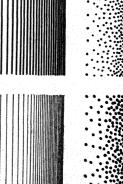
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spaces between black lines or dots may be lost. If placed too close together, dots and lines produce black duction, but that small black dots and small white blotches when the drawing is reduced. Keep the shading rather open. The degree of reduction needs to be Note that thin black lines hold up fairly well in reknown before the drawing is inked in.

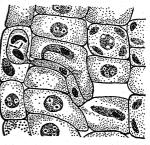
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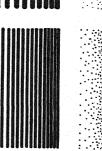
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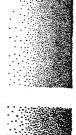
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### INFLUENCE OF GROWTH SUBSTANCES ON GROWTH AND CELL DIVISION IN GREEN ALGAE $^{\scriptscriptstyle 1}$

Melvin A. Brannon and Alfred F. Bartsch

THE FIRST report of the effect of growth substances on algae was made by Yin (1937). In his tests with Chlorella vulgaris he found no measurable increase in the rate of multiplication, but he did find slight increases in size of cells in cultures treated with heteroauxin. At about the same time a report of the effect of heteroauxin on Chlorella miniata, C. pyrenoides (pyrenoidosa?), Cystococcus cohaerans, Oocystis naegelii, and Scenedesmus flavescens was published by Leonian and Lilly (1937). They concluded that heteroauxin is a growth-inhibiting rather than a growth-promoting substance so far as concerns the organisms they studied. Evidence was offered in a preliminary report (Brannon, 1937) of the stimulatory effect of growth substances on Chlorella vulgaris, C. pyrenoidosa, and Oocystis sp. Pratt (1938) offered additional evidence to show that chemical growth substances, including heteroauxin, cause an increase in the rate of multiplication in Chlorella vulgaris. He, however, was unable to confirm Yin's statement that heteroauxin causes an increase in size of cells.

Growth substance studies with vascular plants have probably been emphasized in the past because in these plants correlations, formation of organs, tropisms, and modifications of growth may be studied to better advantage than in algae. In dealing with vascular plants the concentration of growth substance must vary as the solution diffuses through tissues intervening between the point of application and that at which the effects of the applied growth substance are to be studied. This view is supported by Hamner and Kraus (1937) in connection with their histological study of bean plants treated with applications of indole-acetic acid in lanolin. If, instead of using lanolin, the growth substances are injected into the underlying tissues or applied superficially after removing the overlying tissues, the factor of traumatism is introduced. On the other hand, unicellular algae may be immersed in solutions containing known concentrations of growth substance, and the results of such treatment should indicate the effects of the concentration in question. In both cases, however, the concentration of growth

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The Wisconsin Alumni Research Foundation generously supported this work with grants for two years.

substances which enters into the cells is still unknown.

Bonner (1934) has pointed out that the magnitude of growth substance effects is directly related to the hydrogen-ion concentration of the medium in which the growth substance is applied. He concluded from his study with the Avena coleoptile that the growth substance is converted from the form of an inactive salt to an active nondissociated form in the presence of acid buffers. Marmer (1937) also found that the effect of growth substances on the growth of wheat seedlings is related to the pH of the applied solution. She reported that indoleacetic acid is 15,000 times as effective at pH 4.6 as at pH 7.5, indole-butyric acid is 10 times as effective, and indole-propionic acid 20 times as effective. Albaum, Kaiser, and Nestler (1937) found that the penetration of indole-acetic acid into cells of Nitella takes place more rapidly at pH 3.65 than at pH 7.94. On the basis of their results they concluded that the hydrogen-ion concentration affects the degree of dissociation of molecules of growth substance and that heteroauxin enters cells of Nitella in molecular form. Since in algal cultures it is possible to adjust the pH to a definite value, the effects of known concentrations of growth substance may be studied and compared to greater advantage than in the case of vascular plants where the hydrogen-ion relation may become a variable factor.

The present study was undertaken to determine the effect of several growth substances on cell division and cell size in three species of unicellular green

MATERIALS AND METHODS.—Three types of algae which have different growth rates under identical conditions were selected for this study. Chlorella vulgaris Beyerinck was selected as representative of the fast-growing, Coccomyxa simplex (?) of the intermediate, and Mesotaenium caldariorum (Lagerh.) Hansg. of the slow-growing forms. The first named species was selected in accordance with Du Buy's (1936) statement that "it has been proved previously that growth regulators of the auxin type have an effect only on cells which are in the embryonic stage." Went and Thimann (1937) also concluded that "the cells on which the auxins act are of the least differentiated and simplest type." The unicellular algae which possess a stage corresponding to embryonic cells of higher plants are those which, as does Chlorella, reproduce by means of autospores. These spores have exceedingly thin walls and are highly responsive to physical and chemical changes in the medium in which they are immersed. These algae were identified in the Pflan-

[The Journal for April (26: 179-269) was issued May 15, 1939.] American Journal of Botany, Vol. No. 5, May, 1939.

Table 1. Acid concentrations (moles per liter).

Ppm.	Indole- acetic	Naphthalene- acetic	Indole- butyric	Indol <del>e</del> - propionic	Phenyl- acetic
0.10	6.0×10 <sup>-7</sup>	5.5×10 <sup>-7</sup>	4.9×10-7	5.3×10 <sup>-7</sup>	$7.4 \times 10^{-7}$
0.33	$2.0 \times 10^{-6}$	1.8×10-6	$1.6 \times 10^{-6}$	$1.4 \times 10^{-6}$	$2.5 \times 10^{-6}$
0.50	3.0×10-6	2.7×10-6	$2.4 \times 10^{-6}$	$2.6 \times 10^{-6}$	$3.7 \times 10^{-6}$
1.00	$6.0 \times 10^{-6}$	5.5×10-6	$4.9 \times 10^{-6}$	$5.3 \times 10^{-6}$	$7.4 \times 10^{-6}$
2.00	$1.2 \times 10^{-5}$	$1.1 \times 10^{-5}$	$9.8 \times 10^{-6}$	$1.1 \times 10^{-5}$	$1.5 \times 10^{-5}$
3.33	$2.0 \times 10^{-5}$	1.8×10-5	$1.6 \times 10^{-5}$	$1.4 \times 10^{-5}$	2.5×10-5
6.7	$4.0 \times 10^{-5}$	3.6×10-5	$3.3 \times 10^{-5}$	$2.8 \times 10^{-5}$	$4.9 \times 10^{-5}$
10.0	$6.0 \times 10^{-5}$	5.5×10-5	$4.9 \times 10^{-5}$	$5.3 \times 10^{-5}$	$7.4 \times 10^{-5}$
20.0	$1.2 \times 10^{-4}$	1.1×10-4	$9.8 \times 10^{-5}$	$1.1 \times 10^{-4}$	$1.5 \times 10^{-4}$
33.3	$2.0 \times 10^{-4}$	1.8×10-4	$1.6 \times 10^{-4}$	$1.4 \times 10^{-4}$	$2.5 \times 10^{-4}$
100.0	$6.0 \times 10^{-4}$	5.5×10 <sup>-4</sup>	4.9×10 <sup>-4</sup>	5.3×10 <sup>-4</sup>	$7.4 \times 10^{-4}$

zenphysiologisches Institut of Prof. E. G. Pringsheim at Prague.

To assure purity and freedom from contamination by bacteria and fungi, agar-plate cultures were made, and a single clone was used as the source material for all subsequent work.

The same liquid medium was used in all experiments. It consists of: 1.0 g. potassium nitrate (0.0099 M), 0.5 g. calcium sulfate (0.0037 M), 0.5 g. magnesium sulfate (0.0020 M), 0.25 g. tribasic calcium phosphate (0.008 M), 0.01 g. ferric chloride (0.0006 M), and 1000 cc. distilled water. The tri-basic calcium phosphate was first dissolved separately in 100 cc. of distilled water, and the other salts were dissolved together in 900 cc. of distilled water. The solutions were then boiled, filtered, and mixed.

Alpha-naphthalene-acetic acid, (indole-3)-n-butyric acid, indole-3-acetic acid, beta (indole-3)-propionic acid, and phenyl-acetic acid were used as growth substances. When this work was begun in 1935 they were obtained from Dr. P. W. Zimmerman of the Boyce Thompson Institute for Plant Research and later from the Eastman Kodak Company and from Merck and Company.

Aqueous solutions which were used in our first experiments were abandoned for alcoholic solutions (1 per cent in 95 per cent alcohol) following a suggestion of Dr. Zimmerman that water is a less effective solvent and that aqueous solutions are probably unstable. Successive dilutions of the alcoholic solution were made with the culture medium in order to obtain the following concentrations (table 1).

Ten cc. of medium were then transferred to each cotton-stoppered Pyrex test tube, and the media were then sterilized by autoclaving for 30 minutes at 15 pounds' pressure.<sup>3</sup>

Preliminary tests indicated the optimum pH range for controlled growth of the three algae to be from

 $^{2}$  M = Molal.

<sup>3</sup> Crystalline indole-acetic acid was hermetically sealed in a fused glass tube which was then immersed in culture medium and autoclaved. The tube was later broken, and dilutions were made under sterile conditions. The effect on cell division of indole-acetic acid treated in this way was not significantly different from that which was dissolved in medium and autoclaved.

5.5 to 6.5. Consequently the pH of all the media used was adjusted within this range by the addition of hydrochloric acid or of sodium hydroxide. Determinations of pH were made with the aid of a Beckman glass electrode.

An equal volume of inoculum was introduced into each culture tube with a glass-sealed constant-flow burette so constructed that all air coming into contact with the inoculum was first filtered through sterile cotton. The entire apparatus was sterilized by autoclaving, and the inoculation room was sprayed with mercuric chloride solution.

Throughout the duration of each experiment, which in most cases was 12 days, the growing cultures were agitated for 5 minutes in each hour by an automatically timed, electrically operated vibratory rack on which the tubes were placed. Illumination was supplied by a 200-watt electric bulb placed at a distance of 1 meter from the cultures. The thermostatically controlled temperature of the culture room varied between 24° and 27°C., and at any given

Table 2. Agreement between calculated and counted numbers of cells of Chlorella when grown in control media and in media containing 10.0 ppm. of indole-acetic acid. Numbers of cells are expressed in millions per cc. of medium.

	Cont Calculated	rol Counted	Indole-ac Calculated	
Microamps.	number		number	number
79.5	1.8	1.86		
77.5	3.9	3.80	3.9	3.89
76.0	5.1	5.14		
74.5			6.2	6.21
72.5	7.8	7.79		
72.0			8.1	8.03
70.5	9.0	9.12		
70.0		15 1. W	9.3	9.49
67.5			11.0	11.11
66.5		11.61		
64.5	13.2	13.58	13.2	13.39
60.0			17.4	17.89
54.0		24.61	24.4	24.47
48.0	37.2	37.91		
47.5			38.8	39.14
46.0		48.83		

time all the cultures were at the same temperature.

In most cases the cultures were removed from the vibratory rack after a 12-day growth period, and the number of algal cells per cubic centimeter was determined. Because of the large number of cultures to be measured, haemacytometer counts were not always made, but the turbidity measurements were checked against curves calibrated from haemocytometer determinations. Turbidity was measured by determining the light absorption of a 47 mm. layer of culture as indicated by a Photox (Westinghouse Electric Co.) cell and a microammeter. Agreement between turbidity determinations and haemacytometer counts of cells grown in control media and in media containing 10.0 ppm. of indole-acetic acid is shown in table 2.

The experimental results are presented in the graphs and tables which follow. In the graphs the concentrations of the various acids are arranged, in ppm., on logarithmic abscissae; the number of cells, in millions per cc. of culture, are plotted as ordinates. The horizontal line C represents the number of cells, in millions per cc., in the control cultures. The location of each point on the graphs was determined by the average of measurements from at least four replicate cultures.

Results.—Chlorella.—In the first experiment conducted with Chlorella, a single concentration of indole-acetic acid was used, and comparative growth curves were made of the growth substance and control cultures. Series A (fig. 1) includes the control cultures, and series B those growing in media containing 10 ppm. of indole-acetic acid. These two series were grown at culture-room temperatures of 24° to 27°C. for 42 days, and turbidity measurements were made at intervals of 3 days. The graph indicates that from the time of inoculation the cells in the cultures containing the indole-acetic acid were multiplying at a faster rate than those in the control cultures. After 15 days' growth the number of cells in the growth substance series was 224.0 per cent greater than in the control series. After that time the rate of cell multiplication in the control cultures was greater than in those containing the growth substance so that the two curves converged as the growth period increased. The maximum difference in number of cells per cc. between the control and indole-acetic acid cultures occurred between

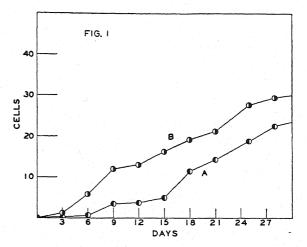


Fig. 1. Growth curve of *Chlorella*. A = Control; B = Series treated with 10.0 ppm. of indole-acetic acid. Number of cells expressed in millions per cc.

12 and 15 days after inoculation. Consequently, in later tests made to determine the optimum concentration of each of the acids, turbidity measurements were made at the end of a 12-day growth period.

In the later tests, 11 concentrations of the five acids were used. The conditions for growth were the same for each test, and all measurements were made at the end of a 12-day growth period. The tests with indole-acetic and naphthalene-acetic acids were conducted at the same time, and the same control cultures were used with both of these test series. The other tests were conducted separately and at different times and consequently had separate controls. The results of these tests are shown in table 3 and in figures 2, 3, 4, 5, and 6.

1. Indole-acetic acid (fig. 2). The optimum concentration of this acid was found to be 10.0 ppm.; and the growth-preventing concentration, 33.3 ppm. The number of cells per cc. at the optimum concentration was 119.0 per cent greater than that of the control. In a concentration as low as 0.10 ppm., the number of cells per cc. was 15.0 per cent greater than that of the control.

2. Naphthalene-acetic acid (fig. 3). The optimum concentration of this acid for cell multiplication was 3.33 ppm., and the growth-preventing concentration

Table 3. Comparison of effect of five different acids upon reproduction in Chlorella after a 12-day growth period. G.P.C. = growth-preventing concentration.

	Millions or	f cells per cc	. of culture	Acid con- centrations in ppm.	Increase, optimum over
Acid	Opt.	0.10 ppm.	Control	Opt. G.P.C.	control
Indole-propionic	9.00±0.21	5.26±0.13	5.20±0.09	6.7 50.0	73.1%
Indole-acetic	$16.00 \pm 0.82$	$8.40 \pm 0.46$	$7.30 \pm 0.15$	10.0 33.3	119.0%
Indole-butyric	$19.20 \pm 1.08$	$7.90 \pm 0.11$	$7.20 \pm 0.09$	6.7 33.3	166.5%
Naphthalene-acetic	$19.90 \pm 0.93$	$7.65 \pm 0.21$	$7.30 \pm 0.15$	3.3 33.3	172.4%
Phenyl-acetic	$19.00\pm0.09$	5.75±0.09	$5.25 \pm 0.13$	33.3 100.0	261.4%

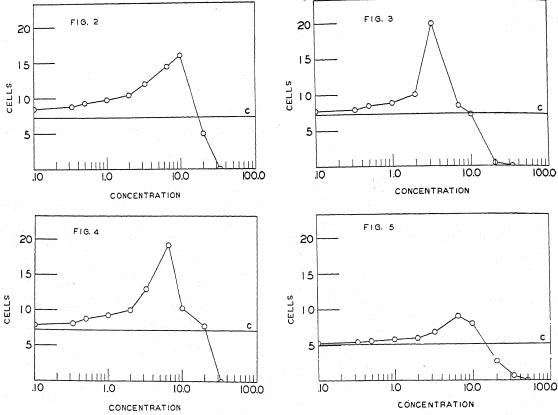


Fig. 2-5. Multiplication of *Chlorella* in several concentrations of different growth substances. Acid concentrations in ppm.; number of cells in millions per cc.; horizontal line, *C*, represents control.—Fig. 2. Indole-acetic acid.—Fig. 3. Naphthalene-acetic acid.—Fig. 4. Indole-butyric acid.—Fig. 5. Indole-propionic acid.

was 33.3 ppm. In the optimum concentration the number of cells per cc. was 172.4 per cent greater than that of the control, and there was no significant stimulation by a concentration of 0.10 ppm.

3. Indole-butyric acid (fig. 4). The optimum concentration for cell multiplication was 6.7 ppm.; the growth-preventing concentration, 33.3 ppm. At the optimum concentration there were 166.5 per cent more cells per cc. than in the control. The stimulatory effect of a concentration of 0.10 ppm. was probably not significant.

4. Indole-propionic acid (fig. 5). The optimum concentration was found to be 6.7 ppm.; the growth-preventing concentration 50.0 ppm. There were 73.1 per cent more cells per cc. in the optimum concentration than in the control, and the effect of 0.10 ppm. on the rate of multiplication was not significant.

5. Phenyl-acetic acid (fig. 6). The optimum concentration was 33.3 ppm.; the growth-preventing concentration, 100 ppm. At the optimum concentration the number of cells per cc. was 261.4 per cent greater than that of the control, and the effect of 0.10 ppm. was not significant.

The order of maximal effect of these five acids is, in decreasing order: (1) phenyl-acetic acid, with an increase of 261.4 per cent over the control; (2) naphthalene-acetic acid, with an increase of 172.4

per cent; (3) indole-butyric acid, with an increase of 166.5 per cent; (4) indole-acetic acid, with an increase of 119.0 per cent; (5) indole-propionic acid, with an increase of 73.1 per cent. The optimum concentration of these acids ranges from 3.33 ppm. for naphthalene-acetic acid to 33.3 ppm. for phenylacetic acid; the growth-preventing concentration ranges from 33.3 ppm. for naphthalene-acetic acid to 100 ppm. for phenyl-acetic acid. Of the five acids, indole-acetic is the only one that caused significant stimulation in a concentration as low as 0.10 ppm. (table 3).

It was reported by Yin (1937) that applications of indole-acetic acid caused increases in the size of cells of C. vulgaris. In the present study, measurement of more than 1,200 cells of C. vulgaris indicated that the mixture of autospores, germinating autospores, and adult cells made it impossible for us to secure reliable data respecting the effect of a growth substance upon the size of cells. In addition, the fact that the cells range from only about 5 to 10 microns in diameter makes accurate measurement extremely difficult. Recently Pratt (1938) found that "heteroauxin was effective in promoting cell multiplication in Chlorella vulgaris, but it had exerted little, if any, influence on the cell size at the end of approximately three weeks." It is possible,

however, that Yin and Pratt were working with different strains of the alga.

A study of *Chlorella* was made to determine the rate of increase in number of cells after a 4-day growth period (A) and after an 8-day period (B)

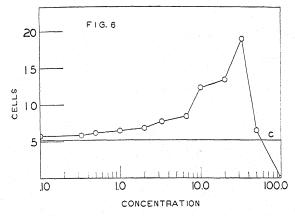


Fig. 6. Multiplication of *Chlorella* in several concentrations of phenylacetic acid. Acid concentrations in ppm.; number of cells in millions per cc.; horizontal line, *C*, represents control.

when the cultures were grown in media containing 11 different concentrations of indole-propionic acid. The results are shown in figure 7 and table 4.

After a 4-day growth period the number of cells per cc. in a concentration of 6.7 ppm. was 16.6 per cent greater than in a concentration of 0.10 ppm. so that different rates of multiplication in different acid concentrations were already evident. During the second 4-day period the number of cells in the optimum concentration of 6.7 ppm. increased 189.7 per cent over that at the end of the first growth period in the same concentration. It can be seen from table 4 that the increase in number of cells per cc. during the second 4-day period over that of the first was greatest in a concentration of 10.0 ppm.; it also shows that the magnitude of increase was progressively less toward the two extremes of concentration. Although the concentration of 6.7 ppm. appears to

be optimum, the number of cells in the next higher concentration (10.0 ppm.) increased 125.0 per cent more rapidly during the second period than did that in the apparent optimum concentration of 6.7 ppm. This indicates that at a later time the highest number of cells would probably be in the higher concentration and that the apparent optimum changes continually until a static cultural condition is reached.

It was found that the response of Chlorella to a growth substance could be both increased and prolonged by agitating the growing cultures, thus facilitating release of autospores from the mother cells and permitting exposure of the autospores to the growth substance. Other effects undoubtedly result from agitation, such as more rapid gas diffusion through the culture medium and the prevention of an accumulation of deleterious substances about the cells. Figure 8 shows the following results of periodic agitation for 12 days: first, the number of cells per cc. was greater in agitated (A) than in nonagitated cultures (B) both in the controls and those containing indole-acetic acid; second, in non-agitated cultures the optimum concentration of indole-acetic acid was 2.0 ppm. with 6,750,000 cells per cc.,

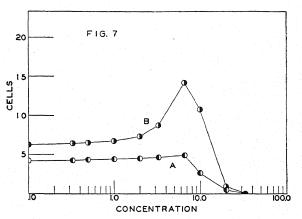


Fig. 7. Multiplication of *Chlorella* in several concentrations of indole-propionic acid after a 4-day (A) and an 8-day (B) growth period. Acid concentrations in ppm.; cells in millions per cc.

Table 4. Effect of indole-propionic acid upon reproduction in Chlorella after 4-day and 8-day growth periods.

	Concentration of indole-propionic acid (ppm.).										
	0.10	0.33	0.50	1.00	2.00	3.33	6.7	10.0	20.0	33.3	100
Days				Millio	ns of ce	lls per	cc. of cı	ılture.			
	(4.20	4.25	4.30	4.40	4.50	4.60	4.90	2.60	0.50)		
4	<b>√</b> ±	± *	±	<b>±</b>	±	<b>±</b>	±	± .	± }	0	0
	0.31	0.09	0.01	0.06	0.02	0.23	0.18	0.01	0.02		
	6.20	6.40	6.50	6.70	7.30	8.80	14.2	10.8	0.80		
8	ñ	<u>+</u>	±	+	<u>±</u>	±	±	±	± }	0	0
	0.49	0.39	0.83	0.09	0.41	0.30	0.08	0.28	0.03		
			Increas	e in nun	aber of c	ells of 2	nd over	1st 4-da	y period.		
	47.6%	50.4%	50.8%	52.2%	62.1%	91.0%	189.7%	314.7%	60.0%		a

whereas when the cultures were agitated, the optimum was 10.0 ppm. with 19,900,000 cells per cc.; and third, the growth-preventing concentration of growth substance in the agitated cultures was higher (40.0 ppm.) than in the quiescent cultures (20.0

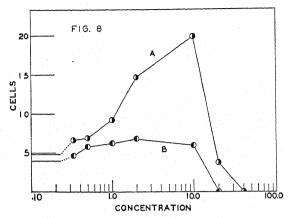


Fig. 8. Effect of periodic agitation upon multiplication in *Chlorella* when grown in media containing several concentrations of indole-acetic acid. A = cultures agitated 5 minutes in each hour for 12 days; B = quiescent cultures. Controls are connected to corresponding series by broken lines. Acid concentrations in ppm.; cells in millions per cc.

ppm.). Hence it is concluded that agitation caused a shortening of the life cycle of the cells of *Chlorella* and also permitted a longer period of growth. It can be seen that the greatest effect of agitation was in the region of optimum acid concentration. The results of this treatment were the same for the five acids.

In studies of the effect of growth substances on vascular plants an interaction has been postulated between the food factor and growth substance factor (Went and Thimann, 1937; Schneider, 1938). It was found (Schneider, 1938) that the addition of both auxin and sugar to the Avena coleoptile, using the section test of Bonner (1933), increased the rate of growth more than the addition of either auxin or sugar alone. Schneider found also that the mag-

nitude of growth increase, when suboptimal concentrations of sugar and growth substance are used, is proportional to the product of the logarithms of the concentrations.

From results obtained by growing cells of Chlorella on agar plates (fig. 9) it appeared that the influence upon the algal cells of the growth substancefood factor interaction is possibly different from that of vascular plants. The colonies shown were selected as typical representatives of each series of cultures. Colonies labeled B were grown for 3 days on agar containing only the inorganic salts used in all the test media, colonies C were grown for the same period on agar containing an addition of 0.10 per cent dextrose, and colonies A were grown on agar containing both 0.10 per cent dextrose and 10.0 ppm. of indole-acetic acid. The effect of 0.10 per cent dextrose upon the size of the algal colonies was as great as or greater than that of the dextrose and heteroauxin together.

Since agar plates are not ideal for physiological experimentation, the test was repeated with liquid media. These media were prepared as follows: (1) containing the inorganic salts alone in the proportions previously described; (2) with an addition of 10.0 ppm. of indole-acetic acid; (3) with an addition of 0.10 per cent dextrose; (4) addition of both 10.0 ppm. of indole-acetic acid and 0.10 per cent

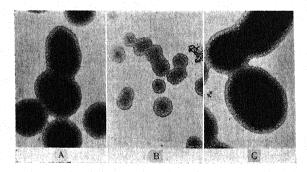


Fig. 9. Colonies of *Chlorella* grown on agar for 3 days. A = agar containing 0.1 per cent dextrose and 10.0 ppm. of indole-acetic acid, B = control, C = agar containing 0.1 per cent dextrose.

Table 5. Reproduction of Chlorella in media containing indole-acetic acid and/or dextrose. Values are expressed in factors (test/control) calculated by statistical methods from triplicate cultures. Millions of cells per cc. for control cultures enclosed in parentheses. C = Control, I = 10.0 ppm. indole-acetic acid, II = 0.10 per cent dextrose, III = 10.0 ppm. indole-acetic acid + 0.10 per cent dextrose, IV = 0.25 per cent dextrose, V = 10.0 ppm. indole-acetic acid + 0.25 per cent dextrose.

	Day		7 <b>S</b>	
	3	6	9	12
C	1.00 (4.23±0.04)	1.00 (7.33±0.17)	1.00 (8.46±0.08)	1.00 (11.0±0.01)
I	$1.47 \pm 0.02$	$1.39 \pm 0.10$	$1.48 \pm 0.13$	1.50±0.05
II	3.18±0.13	$2.37 \pm 0.05$	$2.38 \pm 0.02$	2.09 + 0.01
III	$3.07 \pm 0.21$	$2.18 \pm 0.05$	$2.57 \pm 0.02$	$1.96 \pm 0.12$
IV	2.82±0.03	2.07±0.33	$2.50 \pm 0.01$	2.57 + 0.10
V	$2.30 \pm 0.08$	$2.06 \pm 0.04$	$2.16 \pm 0.08$	$2.40 \pm 0.01$

dextrose; (5) addition of 0.25 per cent dextrose; (6) addition of both 10.0 ppm. of indole-acetic acid and 0.25 per cent dextrose.

Media of the 6 kinds were inoculated with cells of *Chlorella*, and the cultures were maintained under constant cultural conditions for 12 days. The number of cells per cc. was determined for each series of cultures at intervals of 3 days. The results of this test are shown in table 5.

The following indications may be shown by the statistical data expressed above: (1) all the test cultures show marked stimulation over the control for a given column (i.e., after 3, 6, 9, and 12 days' growth); (2) there is no significant difference between the stimulation produced by 0.10 per cent dextrose and that produced by 10.0 ppm. of indoleacetic acid plus 0.10 per cent dextrose for a given column; however, there is a tendency for greater stimulation by 0.10 per cent dextrose alone; (3) there is a marked tendency for greater stimulation by 0.25 per cent dextrose than by 10.0 ppm. of indole-acetic acid plus 0.25 per cent dextrose; (4) there is also a marked tendency for 10.0 ppm. of indole-acetic acid plus 0.10 per cent dextrose to produce greater stimulation than 10.0 ppm. of indoleacetic acid plus 0.25 per cent dextrose during the first 9 days with comparatively lesser stimulation from the ninth to the twelfth day; this suggests that inhibitory effects possibly result from the presence of too high a concentration of dextrose and that the effect of the indole-acetic acid does not become evident until the inhibitory effects of the dextrose are overcome; (5) further evidence for the conclusion that 0.25 per cent dextrose exerts inhibitory effects in the early stages of incubation is shown by the fact that the stimulation produced by 0.10 per cent dextrose was greater than that produced by 0.25 per cent dextrose (with or without the presence of 10.0 ppm. of indole-acetic acid) after 3 and 6 days' growth, with no significant difference after 9 days, and with greater stimulation by 0.25 per cent dextrose after 12 days.

These results are in agreement with those of Schneider (1938) only in so far as the stimulatory effect resulting from a combination of dextrose and growth substance is greater than that produced by the growth substance alone. On the other hand, the stimulation resulting from the combination is less than that produced by the dextrose alone. Since a wide range of dextrose concentrations was not used, the effect of sub-optimal concentrations was not determined. It is possible that with such concentrations the interaction of the two factors would produce a similar effect with algae as with vascular plants.

Coccomyxa.—The second alga tested was Coccomyxa. Because of the slow rate of growth of this species, the period of experiment was lengthened to 24 days—twice that allowed for the tests with Chlorella. The lengthening of the growth period accentuated, in the various cultures, differences which perhaps originated in response to certain uncon-

trollable factors such as small variations in volume of inoculum introduced into each tube, differences in handling glassware prior to starting cultures, or possibly to different rates of gas diffusion through the cotton plugs with which the tubes were stoppered. In spite of such variations, the results indicate that suitable concentrations of the five acids tested do exert a stimulatory effect upon the reproduction of the cells of this species. A single graph (fig. 10) is given to show the reaction of this alga

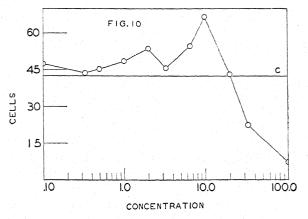


Fig. 10. Multiplication of Coccomyxa in several concentrations of naphthalene-acetic acid. Acid concentrations in ppm.; cells in millions per cc.; horizontal line, C, represents control.

to applications of naphthalene-acetic acid; the results are shown in table 6.

It can be seen that the optimum concentration of naphthalene-acetic acid for reproduction of this species was 10.0 ppm. and that the concentration that prevents growth was not determined, since the cells were still multiplying in a cencentration of 100 ppm. The stimulatory effect of the growth substance resulted in the presence, at the end of the experimental period, of 56.3 per cent more cells than in the control. It is to be noted that this alga is more tolerant of higher concentrations of the growth substances than is *Chlorella*. The optimum concentration of each growth substance for the multiplication of *Coccomyxa* is also higher than that of the same growth substances for multiplication of *Chlorella*.

No evidence was found to indicate that any of the growth substances exerts any influence on the size of cells of Coccomyxa.

Table 6. Influence of naphthalene-acetic acid upon reproduction in Coccomyxa. G.P.C. = growth-preventing concentration.

Millions of cells per cc.				Concentra tion ppm.	
Control	Optimum	Increase 0.10 ppm.	Opti- mum	G.P.C.	
42.6±2.98	66.6±3.73	56.3% 47.4±1.93	10.0	>100	

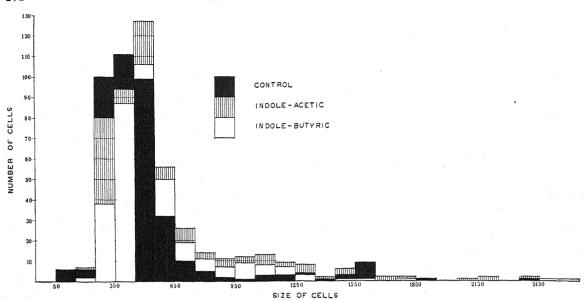


Fig. 11. Comparison of cell sizes of *Mesotaenium* when grown in control media and in media containing either 0.10 ppm. of indole-acetic acid or 0.10 ppm. of indole-butyric acid. Cell sizes expressed in relative volumes. One cell in indole-butyric acid, not shown in figure, reached a size of 5,250 cu. units.

Mesotaenium.—In tests conducted with Mesotaenium the results at the end of a 36-day growth period indicated that each of the 11 concentrations of the 5 growth substances is either inhibitory or at least does not stimulate reproduction. Consequently, an experiment was conducted to determine if any effect upon the size of cells is produced.

Cultures of the alga were made in control media and in media containing 0.10 ppm. of indole-acetic acid in one series and a like concentration of indole-butyric acid in another. The cultures were grown for 4 days before cells were withdrawn for measurement. The cells were measured by the method of Sherbakoff (1927), in which cover-glass smears are placed on thin sheets of agar on microscopic slides, the agar sheet preventing movement of the cells while being measured. A microscope was arranged so that the image, enlarged to a diameter of 100 cm., could be projected on a sheet of white paper 100 cm. square. Outlines of all the cells in any field were drawn on the paper and measured. A total of 1,229 cells was measured and their volumes calculated.

Statistical analysis of the data obtained in this way indicates that there was no significant increase in cell size following treatment with either indole-acetic acid or indole-butyric acid. It is shown by figure 11, however, that there is a tendency for cells treated with the two acids to attain greater relative volumes than those not treated. The figure is arranged as follows: (1) cell volumes are arranged in classes with value intervals of 100 cu. units; (2) the length of a given column represents the number of cells falling into the class in question; (3) the longest of the three columns for a given class is placed in the background and the shortest in the foreground.

Discussion.—Inasmuch as the results reported in this paper show variations from reports of previous workers, it becomes necessary to make a comparison of the techniques employed.

The research of Leonian and Lilly (1937) was based upon a study of "about one hundred different fungi, seven different green algae and corn seedlings." They say in their summary that without a single exception the "higher" concentrations of heteroauxin proved toxic and that the "lower" concentrations failed to produce any stimulation of the algae. The results reported here relative to the toxicity of higher concentrations of heteroauxin (33.3 ppm. and higher) are in entire agreement with toxic results reported by them.

Their conclusion that heteroauxin is a growth-inhibiting rather than a growth-promoting substance has not been confirmed by our experiments. This disagreement is probably due to the following factors: (1) there may have been a widely different pH factor employed in the two studies; no mention was made of the pH used in their tests; (2) they used 0.5 per cent dextrose in their culture media, whereas in the present study it was found that a combination of one-fifth that concentration with 10.0 ppm. of indole-acetic acid produces less stimulation in *Chlorella* than the dextrose alone. It should be men-

<sup>4</sup> In attempting to explain the differential effects produced by dextrose and by a combination of heteroauxin and dextrose, it was found that no qualified conclusion can be made at the present time. Consequently, the following suggestion is offered as a possible explanation. The growth of algae in solutions containing sugar is at least partially heterotrophic, as shown by the experiments of Emerson (1929). It seems possible, then, that the effect of heteroauxin on such plants—namely, inhibition—might be superficially of the same nature as that produced by heteroauxin on fungi which are normally heterotrophic.

tioned in support of this second point that Yin's (1937) report that heteroauxin does not stimulate reproduction in *Chlorella* was based on cultures grown in media which contained sugar, as is indicated by the pertinent literature (Emerson, 1929). On the other hand, the media used in the experiments reported by Pratt (1938), who found, like the present authors, that indole-acetic, indole-butyric, and indole-propionic acids act as stimulators for *Chlorella*, contained no sugar except when the effect of sugar itself was being tested.

The differences in effect of the growth substances on Coccomyxa and Chlorella are possibly due to the facts that the cells of the former are surrounded by a gelatinous matrix and that they reproduce by division into only two cells. These facts may explain the slower rate of growth and the complications which resulted in all experiments involving this species.

From tests which were conducted with Chlorella and Mesotaenium it became apparent that under normal conditions the cells of the former reproduce much more rapidly than those of the latter. The methods of reproduction involved illustrate the reason for this difference. The cells of Chlorella develop and liberate a set of four or more autospores at intervals of about one hour, whereas the cells of Mesotaenium divide into two once in about 12 hours -that is, Chlorella reproduces about 25 or more times as fast as Mesotaenium. It has been pointed out, with support from the work of Pratt (1938), that applications of heteroauxin that stimulate reproduction in Chlorella cause no apparent effect upon the size of cells. This was found to be true of 12-day-old cultures in which the heteroauxin had not been entirely inactivated as indicated by experiments in which the cultures were centrifuged and the supernatant liquid autoclaved and reinoculated. It has been shown by Yin (1937), however, that heteroauxin did affect the size of cells in his cultures. There are two possible reasons for this disagreement: first, different culture media were used in the three studies; and second, different strains of *Chlorella* may be involved.

### SUMMARY

The effects of five monobasic organic acids upon reproduction and cell size in three species of unicellular green algae have been investigated.

Suitable concentrations of naphthalene-acetic, indole-acetic, indole-butyric, indole-propionic, and phenyl-acetic acids, applied in sugar-free inorganic culture media, stimulated reproduction in *Chlorella vulgaris* and *Coccomyxa simplex* but not in *Mesotaenium caldariorum*.

The five acids exerted no measurable influence upon the size of cells in either Coccomyxa or Chlorella, but indole-acetic and indole-butyric acids caused a tendency for cell enlargement in Mesotaenium.

A possible explanation is offered for the different results obtained with the three species of algae.

Evidence is presented to indicate that the interaction of the growth substance factor and the food factor for algae is probably of a different nature from that reported for vascular plants.

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### THE SHAPE OF COMPRESSED LEAD SHOT AND ITS RELATION TO CELL SHAPE <sup>1</sup>

James W. Marvin

Studies of the shape of undifferentiated cells as they occur aggregated into tissues have heretofore often emphasized the rôle of surface tension as a significant physical factor in determining the form of such cells. But, pressure relations between cells, such as parenchyma, aggregated without large intercellular spaces, are also important in shape determination. Experiments were therefore devised for using spheres in a physical system in which surface tension is reduced to a minimum. Small lead shot of uniform diameter were compressed in a cylinder until all the interstices were eliminated, and the shapes of the polyhedra produced by such compression were studied.

Early investigators were impressed with the angular and frequently hexagonal appearance of cells in cross section, and Kieser (1815), considering the three-dimensional shape of parenchymal cells, believed them to be fundamentally rhombic dodecahedra. These figures, with twelve quadrilateral faces of equal size, each have six tetrahedral and eight trihedral angles. Rhombic dodecahedra of uniform size will aggregate to fill space without interstices, and this type of polyhedron may be produced by the compression of uniform spheres regularly stacked, twelve in contact with a central one.

Later, the work of Berthold (1886) and Errera (1887) emphasized the importance of surface tension in cellular systems. Lord Kelvin (1887), after experimenting with liquid film systems, suggested an orthic tetrakaidecahedron as an economical surface-volume configuration having trihedral angles and permitting the division of space without interstices. This figure has eight hexagonal and six square faces; all the edges of the faces are equal; and the surface area of the figure is slightly less than that of a rhombic dodecahedron of equal volume. The orthic tetrakaidecahedron has trihedral, but no tetrahedral angles. The six tetrahedral angles which occur in the rhombic dodecahedron are unstable in a liquid film system.

The first extensive studies of cell shape in three dimensions are the outstanding contributions of Lewis (1923, 1925, 1928, 1930, 1933). He found that cells in undifferentiated tissues in a number of plants and animals are fundamentally fourteen sided, sometimes approaching in shape the orthic tetrakaidecahedron.

METHOD.—In the studies outlined below lead shot of two diameters, 1.27 mm. and 2.54 mm., were used. They were examined under a binocular dissecting microscope, and the asymmetrical ones were discarded. The shot were compressed by means of a

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It is a pleasure to acknowledge the stimulating suggestions and advice of Professor E. B. Matzke under whose direction this work was carried out.

steel plunger in a steel cylinder with a bore 25 mm. in diameter and 75 mm. long.<sup>2</sup>

After compression the shot were studied under a binocular dissecting microscope by means of a mechanical manipulator. This instrument consists of two curved steel needles fitted to a pair of mechanical forceps which can be made to open and close. The forceps are in turn attached to a system of geared adjustments in such a manner that the needles, after picking up a lead ball, can be rotated on a horizontal axis or moved up and down or from right to left in the horizontal field of the microscope. With this device it was possible to study all the faces of a polyhedron in sequence. With slight modification, this manipulator can also be used in studying the shape of cells (Marvin and Matzke, 1939).

To aid in determining the effect of the position of a shot in the cylinder on its number of faces, an ocular guide was constructed. It consisted of two concentric rings so placed that the cross sectional area of the cylindrical mass of shot was divided into three regions. These regions were of approximately equal radial dimension and were about 4.17 mm. wide.

Data.—In the first experiment, shot 2.54 mm. in diameter were used. These shot were poured into the cylinder and compressed at pressures varying from 1,000 to 35,000 pounds. As the result of several trials, 35,000 pounds pressure on the plunger, when applied at the rate of 0.1 inch per minute, was found sufficient to eliminate all the interstices between the shot, to cause them to aggregate, and to form a cylinder of coherent shot whose individual members were easily separated.

The results of these compressions are given in table 1, figures 1-7, and figure 9. Figures 1-7 are photographs of the shot as they appear in the cylinder after compression. Figure 1 shows that flattening occurs at the points of contact as the result of 1,000 pounds pressure. A pressure of 2,500 pounds

Table 1. The average number of contacts observed on lead shot compressed at different pressures.

Pressure Nu	ımber of shot	Average number of contacts
1,000 lbs.	100	8.41
2,500 lbs.	100	8.55
5,000 lbs.	100	10.97
7,500 lbs.	100	11.61
10,000 lbs.	100	12.91
22,500 lbs.	100	13.62
35,000 lbs.	100	14.16

<sup>&</sup>lt;sup>2</sup> These compressions were made possible by the generous and helpful cooperation of Mr. J. N. Kenyon of the Research Laboratories of the Department of Civil Engineering of Columbia University.

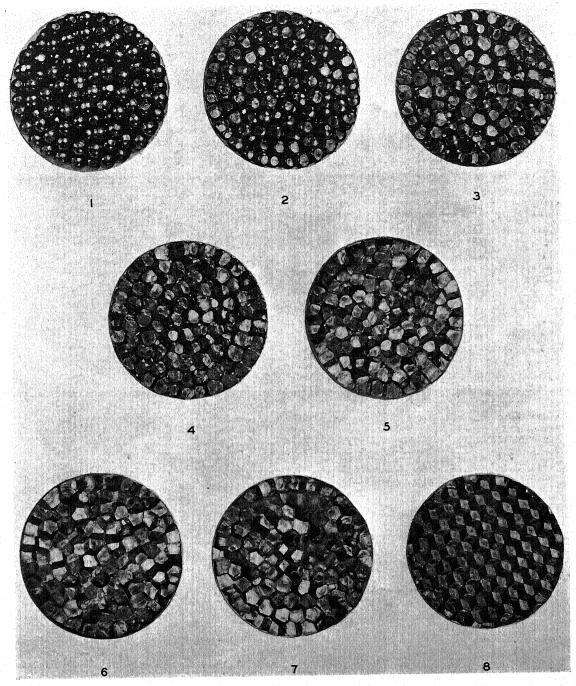


Fig. 1-8.—Fig. 1-7. Photographs of the effect of different pressures on the compressed lead shot of the larger size used (2.54 mm. in diam.).—Fig. 1. 1,000 pounds pressure.—Fig. 2. 2,500 pounds pressure.—Fig. 3. 5,000 pounds.—Fig. 3. 5,000 pounds.—Fig. 4. 7,500 pounds.—Fig. 5. 10,000 pounds.—Fig. 6. 22,500 pounds.—Fig. 7. 35,000 pounds.—Fig. 8 shows the appearance of the shot after compression when stacked in the arrangement to produce rhombic dodecahedra. ×9/5.

produces some faces larger than those seen in figure 1, while new contacts resulting from the additional pressure have produced small faces (fig. 2). This introduction of new faces, from additional contacts due to increasing the pressure, also occurs in figures 3, 4, 5, and 6, while in figure 7 all the

spaces between the shot have been eliminated, and no new contacts can occur. The number of facets has reached its maximum.

Table 1 gives the average number of faces observed on one hundred shot, the number examined at each pressure. These shot were selected at ran-

dom from the cylinder, but they do not include any from the peripheral layer. After being submitted to 1,000 pounds pressure, the shot had an average of 8.41 contacts; at 2,500 pounds, the average was 8.55 contacts per shot, slightly more than that observed at 1,000 pounds. With each increase in pressure, there was an increase in the average number of contacts—namely, at 5,000 pounds, an average of 10.97 contacts, at 7,500 pounds, an average of 11.61, at 10,000 pounds the average was 12.91, and at

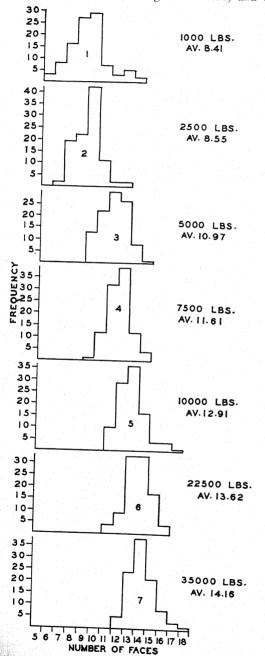


Fig. 9. Histograms of the number of faces on lead shot compressed at varying pressures.

22,500 pounds it was 13.62. At 35,000 pounds all the spaces between the shot were eliminated, and the polyhedra produced by this compression averaged 14.16 faces. This has been previously reported in summary form (Marvin, 1937).

Figure 9 presents the range in the number of faces on the shot examined at each pressure. For figure 9, no. 1, the range is from shot with five contacts to those with thirteen, with a poorly defined mode at 9 contacts per shot. At 2,500 pounds the range becomes somewhat narrower, six to twelve contacts per shot, with a well-defined modal class at nine (fig. 9, no. 2). As the pressure increases up to 35,000 pounds, there is a marked increase in the number of contacts per shot, with the range in the number of contacts remaining very nearly the same at each succeeding pressure—namely, six or seven. The range for each histogram does not include the range for the series, but as the pressure is increased, the relative position of the histograms shifts in the direction of more contacts per shot.

Experiment 1 indicates that after shot are poured into a cylinder, there are approximately eight points of contact between shot (these contacts may be called "primary"), and as the spaces between the shot are eliminated by pressure, "secondary" contacts occur—that is, additional contacts between shot not in contact at first. With increasing pressure, the number of contacts increases to about 14, when all the interstices are eliminated.

In the second experiment, the large shot, 2.54 mm. in diameter, were placed in the cylinder one at a time in the so-called "cannon ball" arrangement. Here the shot in the first and succeeding layers were placed in parallel rows, and except at the periphery each shot was in contact with 6 others in its own layer. The second layer of shot was placed on the first in such a manner that each shot was supported by three from the first layer, and each member of the third layer was in turn supported by three members of the second layer. Thus it may be seen that each shot (except those at the periphery) in any given layer was in contact with six others of the same layer and was also in contact with three members of the layer above and three of the one below, making twelve contacts in all. The cylinder was filled with shot stacked in this manner and submitted to 35,000 pounds pressure to eliminate all the interstices.

An examination of these shot after compression showed them to be dodecahedra. Perfect symmetry was not achieved, however, for the unilateral pressure caused a slight flattening of the figures and certain of the faces were smaller than the others. Here twelve contacts between shot occurred before compression, and twelve faces were present after all the spaces had been eliminated (fig. 8).

Experiments 3, 4, and 5 are similar in some respects and will be considered together. In an attempt to determine the effect of the position of a shot in the cylinder on the number of its faces, the cross-sectional area of the cylindrical mass of shot

Table 2. Number of faces on lead shot poured into a cylinder and compressed to eliminate all the spaces between the shot. Discussion in the text.

				. N	Juml	oer o	f fac	es p	er sh	ot					Mean
		8	9	10	11	12	13	14	15	16	17	18	Number of shot	Mean	Zones B, C, D
Experiment 3	Zone A  Zone B	1	2	13	11	7	1 9	2 25	 16	5			37 63	10.86	
Small shot shaken down	Zone C Zone D	•••	••	••	1 2	12 10	22 20	26 31	21 21	11 14	7 2		100 100	14.15 14.09	14.15
Experiment 4 Small shot not shaken down	Zone A Zone B Zone C	1 	2 	11  1	15 2 4	9 4 5	1 14 14	23 33	6 28	6 10	6 5		39 61 100	10.82 14.13 14.23	14.18
Experiment 5 Large shot not shaken down	Zone D Zone B Zone C	1 	4	10 	3 10 	9 3 1 4	17 1 6 11	30 1 6 14	26  2 11	11  2 3	3  1 2	1	100 30 18 46	14.17 J 10.57 14.05 14.17	· 14.16
Summary Experiments 3, 4, and 5	Zone D Zone A Zones B, and D	 3 C,	8	34	36 12	19 50	7 3 120		139	3  65		  2	36 106 624	14.19 J 10.75	

was divided into three regions of equal radial dimensions in each experiment by using the ocular guide previously described. The peripheral layer of the outermost of these three regions was designated as zone A; the remainder of this outermost region was called zone B; the middle region constituted zone C, while the innermost of these regions, containing the shot in the center of the cylinder, comprised zone D. Experiments 3, 4, and 5 differ in the size of the shot used and in the treatment of the shot after being poured into the cylinder. In Experiment 3, the small shot were shaken down after being poured into the cylinder to determine whether or not the number of initial contacts would be changed, thereby possibly altering the number of faces on the shot after compression. In Experiment 4 the small shot were poured into the cylinder but were not shaken down. For Experiment 5, shot 2.54 mm. in diameter, twice the size of those used in Experiments 3 and 4, were used. These large shot were poured into the cylinder and compressed without being shaken down.

One hundred shot were examined from each of zones C and D in Experiments 3 and 4, and one hundred from zones A and B combined. Smaller numbers were studied in Experiment 5. In table 2 the average number of contacts obtained for the shot from the four zones in each experiment are listed. A statistical treatment of the data is given in table 3. In figure 10 frequency histograms are presented showing the range in the number of contacts observed in Experiments 3, 4, and 5, and also a summary of those data.

In Experiment 3 (table 2) in which small shot 1.27 mm. in diameter were poured into the cylinder and shaken down, 37 peripheral shot and 63 from zone B were examined. The peripheral shot had an average of 10.86 contacts; those in zone B, 14.25. One hundred shot from zone C had an average of

14.15 faces, and one hundred shot from zone D averaged 14.09 contacts per shot.

The ranges in the number of contacts for the shot studied from the four zones in Experiment 3 are presented in figure 10. The peripheral layer, 1-A, includes shot with 8 to 14 contacts—a range of 7, with the modal class at 10. Those in zone B, 1-B, have a range of 6, from 12-sided to 17-sided shot. Zones C, 1-C, and D, 1-D, include seven kinds of polyhedra, 11-sided to 17-sided. The last three groups, 1-B, 1-C, and 1-D, all have modes at 14. Thus, zones B, C, and D have very nearly the same range and the same modal class. Their histograms are similar in shape. The histogram of the peripheral shot, 1-A, shows a distribution and a modal class quite different from those of 1-B, 1-C, and 1-D.

In Experiment 4, the small shot, 1.27 mm. in diameter, were poured into the cylinder and compressed without being shaken down. From table 2 it is evident that 39 peripheral shot and 61 shot from zone B were examined. The average for the peripheral shot was 10.82 contacts, while that for zone B was 14.13. The averages for zones C and D were 14.23 and 14.17, respectively.

As shown in figure 10—2A, 2B, 2C, and 2D—the histograms of the data in Experiment 4 are similar to those of Experiment 3—1A, 1B, 1C, 1D. The peripheral shot have a markedly different range and mode from the shot in zones B, C, and D, while zones B, C, and D all show nearly the same range and have the same modal class—namely, 14.

The large shot, 2.54 mm. in diameter, were used in Experiment 5. They were poured into the cylinder and were compressed without being shaken down. In this experiment, in which 30 shot were examined from the peripheral layer, there was an average of 10.57 contacts per shot. From zone B, 18

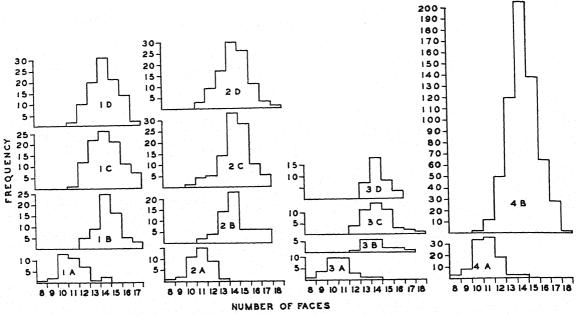


Fig. 10. Histograms of the number of faces on lead shot in Experiments 3, 4, and 5, 1A, 1B, 1C, 1D, histograms for zones A, B, C, and D, respectively, in Experiment 3; 2A, 2B, 2C, 2D, histograms for zones A, B, C, and D, respectively, in Experiment 4; 3A, 3B, 3C, 3D, histograms for zones A, B, C, and D, respectively, in Experiment 5; 4A, summary histogram for zone A in Experiments 3, 4, and 5; 4B, summary histogram for zones B, C, D in Experiments 3, 4, and 5.

shot were examined, and these averaged 14.05 contacts. The 46 shot examined from zone C and the 36 from zone D had averages of 14.17 and 14.19, respectively.

The histograms of the shot studied in this experiment are shown in figure 10—3A, 3B, 3C, and 3D. A range of 7 in the number of faces, 8–14, was observed on the peripheral shot (3A), while the shot from zone B vary in contacts from 12–17, a range of 6. In zone C the spheres were compressed into polyhedra having 12 to 18 sides, a range of 7, and in zone D the range was 4, from 13–16. In Experiment 5 as in Experiments 3 and 4, the peripheral shot have a range and a modal group markedly different from those of the other layers. The range is nearly the same and the modal class is the same for all the deeper layers.

From table 2 it is likewise evident that the averages for zones B, C, and D in each experiment are similar. And further, the averages of these inner zones were essentially the same in Experiments 3, 4, and 5. The data were therefore treated statistically to test the significance of their differences. The ratios in table 3, when interpreted in the light of a table of the areas of the normal probability curve, indicate the chances that the true difference between the means is as great or greater than the observed differences. For approximate analysis a ratio greater than three may be accepted as significant, while one appreciably less than three may be considered not significant. This principle was fol-

<sup>3</sup> It is with pleasure that the helpful suggestions of Professor F. E. Croxton of Columbia University in the statistical treatment of these data are gratefully acknowledged.

Table 3. A statistical treatment of the data in table 2.

	Ratio	
Experiment 3—Zone A and Zone B	13.40	Significant
Experiment 3—Zone B and Zone D	.79	Not significant
Experiment 4—Zone A and Zone B	12.26	Significant
Experiment 4—Zone B and Zone C	.43	Not significant
Experiment 5—Zone A and Zone B	9.21	Significant
Experiment 5—Zone B and Zone D	.47	Not significant
Experiment 3—Zone A and Experiment 5—Zone A	.94	Not significant
Experiment 3—Zones B, C, D and Experiment 4—Zones B,		Ğ
C, D	.25	Not significant
Zone A, Experiments 3, 4, and 5, and Zones B, C, D, Ex-		· ·
periments 3, 4, and 5	24.43	Significant

Ratio: the difference between the two means divided by the standard error of the differences between the two means.

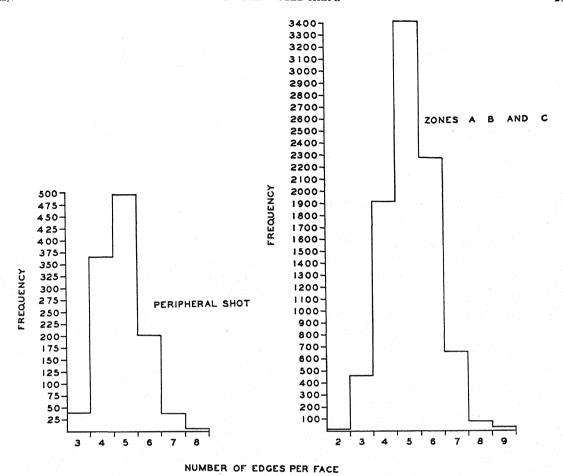


Fig. 11. Histograms of the number of edges per face on the lead shot in Experiments 3, 4, and 5.

lowed here, and it will be observed that all ratios are either much greater or much smaller than 3.

In Experiments 3, 4, and 5 the mean for the peripheral shot in each experiment, when compared with the mean for the layers of zone B in the same experiment, was significantly different; this indicates that the wall of the cylinder has a marked effect on the number of contacts observed for the peripheral shot. Similarly, when the two extreme means for the layers of zone B and for zones C and D (i.e., table 2, Experiment 3, zone D—14.09—and Experiment 3, zone B—14.25) were compared in each experiment, these differences were found not significant. It appears, therefore, that, except for the peripheral shot, the radial position of a shot in the cylinder does not influence the number of contacts it will show after compression.

A comparison of the high and the low means obtained for the peripheral shot in Experiments 3, 4, and 5 showed that here the difference between the two means was not significant. Therefore, using large shot or shaking the shot after it was poured into the cylinder had no significant effect on the number of faces on the peripheral shot. Similarly, the shot from the inner layers comprising zones B, C, and D in these three experiments were compared,

and it was found that shaking the shot after they were poured into the cylinder or using large shot (twice the diameter of the small shot) had no significant effect on the number of contacts produced by compression.

The results for Experiments 3, 4, and 5 were grouped and, as seen in table 2, the 106 peripheral shot had an average of 10.76 contacts, while the 624 inner shot averaged 14.17 contacts or faces.

The frequencies of the kinds of faces found on the compressed lead shot are presented in figure 11 and in table 4. The first histogram (fig. 11) presents the totals of the kinds of faces found on the peripheral shot in Experiments 3, 4, and 5. The totals of all the kinds of faces found on shot from zones B, C, and D in Experiments 3, 4, and 5 are shown in the second histogram. The faces on the peripheral shot range from triangular to octagonal, with pentagonal most common, quadrilateral next, and then hexagonal. The number of pentagonal faces is only slightly less than half the total number of faces of all kinds; there are 495 pentagonal faces and 1141 faces in all. A study of the frequency histogram of the number of faces on shot from zones B, C, and D shows a range in the number of edges on a face from 2 to 9, with pentagonal faces most common, hexago-

Table 4. The frequency of occurrence of the kinds of faces on the 106 peripheral shot and the 624 inner shot examined.

		iments 3, 4 A (outer l		Experiments 3,4, and 5 Zones B, C, and D (inner layers)					
Number of edges per face	Frequency	Ratio on basis of 10.75	Approximate ratio	Frequency	Ratio on basis of 14.17	Approximate ratio			
2	0	0.00	0	1	0.00	0			
3	40	.38	0	465	.70	1			
4	364	3.43	3	1921	3.12	3			
5	495	4.66	5	3420	5.53	6			
6	200	1.89	2	2276	3.68	4			
7	37	.35		666	1.13	1			
8	5	.04	0	84	.14	0			
9	0	0.00	0	7	.01	0			

nal faces next, and quadrilateral faces third. A face with two edges is possible only if the edges are curved, and this is not common, for in general the edges are straight (fig. 7). The number of pentagonal faces is approximately 3/8 of the total number of faces, since there are 3420 pentagonal faces in a total of 8840. Thus, both in the peripheral layers and in zone B as well as in zones C and D, pentagonal faces are most common.

Table 4 gives the frequency of occurrence of each kind of face, and also a ratio which shows the proportionate occurrence of each kind of face in the peripheral layer, zone A, in Experiments 3, 4, and 5, as well as for zones B, C, and D.

A study of the number of trihedral and tetrahedral angles on 106 peripheral shot in Experiments 3, 4, and 5 revealed 1834 of the former and 12 of the latter. Thus, the proportion of tetrahedral to trihedral angles is 1 to 153. Similarly, in the 624 shot from zones B, C, and D there were 14,866 trihedral angles and 159 tetrahedral angles, or 1 tetrahedral to 93 trihedral angles.

Discussion.—In Experiment 1, the irregular appearance of the lead shot is explained by their random and uneconomical space-filling arrangement. A pressure of 1,000 pounds caused a flattening of the shot at the points of contact between adjacent shot, and a study of these individuals showed the contacts to be irregularly distributed. As the pressure was increased, these first-formed contacts became the large faces with a relatively large number of edges, and when compressed further, new contacts occurred which produced faces smaller than those formed first. There seems to be a direct correlation between the distance between the centers of any two shot which are in direct contact, or which will eventually be in contact after compression, and the size of the resulting faces. In other words, the faces produced from primary contacts normally will be larger than those from contacts induced by pressure.

In contrast with these results are those of Experiment 2 in which shot were placed in the cylinder in cannon-ball fashion and compressed. No new contacts or faces were introduced, and 12 faces,

representing the 12 original contacts, were produced. Shot poured into the cylinder, as in Experiment 1, had fewer than 12 initial contacts, and, after compression, the introduction of new faces from secondary contacts gave a total of more than 12 faces.

In Experiments 3, 4, and 5 the radial position of a shot in the cylinder, except in the peripheral layer, did not cause a significant change in the number of faces produced by compression. Also, neither shaking the shot down after pouring nor using large shot in the cylinder significantly affected the number of faces resulting from the pressure.

In this physical system, in which the lead shot were submitted to pressure in a cylinder whose diameter is approximately 20 times that of the shot, a ratio was established similar to that existing in some plants between the diameter of the pith and that of individual pith cells. In zones B, C, and D in Experiments 3, 4, and 5 the average number of contacts in each case was slightly more than 14. Lewis (1937) has suggested that this excess above 14 is due to the curvature of the tube, the increasing circumference peripherally tending to allow additional contacts. Statistically 14.17 is significantly different from 14, under the conditions here described and if 14 is an ideal figure subject to no variation. This conclusion is based on the relatively large number (624) in our sample. Each of the individual means in table 2-i.e., 14.25, 14.15, 14.09, 14.13, etc.—based on a smaller number of shot is not significantly different from 14 statistically. An average of 14 faces might be realized under ideal conditions. However, the experiments here described are subject to certain limitations such as the unilateral pressure and the relatively large diameter of the shot compared with the diameter of the cylinder, so that the curvature of the cylinder might well have an effect on the number of contacts. Bearing such factors in mind, the striking feature is perhaps not the difference from 14, which is barely distinguishable, but the close approximation to 14 that was actually achieved. When compared with cell data (Lewis, 1923) in which the cells observed had a range from six to twenty contacts, the averages for the 624 shot (14.17) and for the 100 cells (13.96) are not significantly different.

Although the average number of contacts was 14.17, the orthic tetrakaidecahedron, with its 8 hexagonal and 6 square and no pentagonal faces, was not realized in the shot, since the latter had many more pentagonal than either hexagonal or quadrilateral faces. Indeed it has not been found completely realized in plant or animal tissue, in which pentagonal facets are likewise abundant, though there is a recognizable approach to the orthic tetrakaidecahedron in certain cells (Lewis). Thus, the similarity between the shape of cells which are considered deviations from the orthic tetrakaidecahedron and the lead shot exists in the number of contacts and the relatively few tetrahedral angles of which the orthic tetrakaidecahedron has none.

Lewis (1928), studying the shape of the columnar epidermis of cucumber, frequently found these cells to have eleven sides, with 10 contacts between cells and one exposed face. Such cells would have 6 lateral contacts, 4 basal contacts, and one face exposed at the surface. In the study set forth here, 106 peripheral shot averaged 10.75 contacts, and some of the individuals observed had a pattern similar to that described for the epidermal cells. In Experiments 3, 4, and 5 the peripheral layer in each case had slightly fewer than 11 faces. Lewis (1937) again ascribes this difference to the curvature of the cylinder and "the diminished circuit of the row next within" the outermost layer. The average of 10.75 found in the shot is not significantly different statistically from 11 under the conditions of those experiments.

In the experiments on the lead shot presented above, surface tension and the biological factors affecting cells in tissues have not been active, although they are highly important in living organisms. However, the striking agreement in number of contacts between cells in tissues (Lewis) and the lead shot compressed to eliminate interstices indicates that pressure relationships are of prime importance in cell shape determination.

It seems very probable, furthermore, that the intercellular spaces between cells in certain tissues may result as much from pressure relations as from physiological necessity. Cells subjected to relatively low pressure—not necessarily external—may well tend to be rounded and have an abundance of intercellular spaces, while between those strongly compressed, spaces may be small or lacking.

#### SUMMARY

Pressure relations between parenchymal cells have not been emphasized in studies of their shapes. Small lead shot of uniform diameter were compressed, and then examined, to determine the effect of pressure on uniform spheres in a system in which biological factors were not active and surface tension was reduced to a minimum.

Pressures varying from slight to sufficient for the elimination of all the spaces between shot poured into a cylinder gave varying numbers of contacts, from 8.41 with slight pressure to 14.16 when all the spaces had been eliminated. The last figure approximates very closely the average of 13.97 contacts which Lewis found for 250 cells in undifferentiated

The peripheral shot in the cylinder averaged 10.75 contacts per shot, not very different from the 11sided cells of cucumber epidermis studied by Lewis. The 624 shot from the layers other than the peripheral, zones B, C, and D, had an average of 14.17 faces per shot.

The radial position of a shot inside the peripheral layer in the cylinder had no effect on the number of contacts.

Shaking the shot down after they were poured into the cylinder or using shot of twice the diameter had no appreciable effect on the average number of contacts.

The compressed lead shot are not orthic tetrakaidecahedra with 8 hexagonal and 6 square faces, for pentagonal faces are most common, and triangular, heptagonal, octagonal, and nonagonal, as well as quadrilateral and hexagonal faces occur.

The compressed lead shot, like Lewis' cells, had

relatively few tetrahedral angles.

The abundance of intercellular spaces between parenchymal and possibly other cells may result as much from pressure relations between the cells as from physiological necessity.

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# VOLUME-SHAPE RELATIONSHIPS IN LEAD SHOT AND THEIR BEARING ON CELL SHAPES <sup>1</sup>

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Previous work has shown marked and significant similarities in three-dimensional shapes between cells in undifferentiated tissues and compressed lead shot of uniform diameters. Since cells, even in the same tissue, are never identical in size and frequently not uniform, an examination has been made of the shapes of lead shot of two diameters and volumes, compressed so that all interstices were eliminated. The large and small shot were mixed, to simulate cellular conditions in living organisms. A study of the patterns so produced should throw light on the mechanics of cell shape determination.

In 1887 Lord Kelvin wrote a philosophical essay in which he conceived of the division of space into polyhedra each having fourteen faces, eight hexagonal and six square; such figures he called tetrakaidecahedra. Influenced by the earlier work of Plateau (1873) on soap films, he was thinking especially in terms of stability of angles, economy of surface with reference to volume, and stackability. The biological implications of Lord Kelvin's deductions were entirely unappreciated for thirty-six years, until Lewis (1923) published the first of his searching investigations on the three-dimensional shapes of massed cells. These observations have since been extended to diverse kinds of tissues. Considering especially the more undifferentiated tissues of those that Lewis (1923, 1925, 1933) has studied, one hundred elder-pith cells, one hundred human fat cells, and fifty precartilage cells of the tadpole have an average of 13.97 faces—a remarkably close approximation to a fourteen-sided figure. The faces were not exclusively hexagonal and square, however, as would be the case if Lord Kelvin's tetrakaidecahedron were fully realized.

Recently Marvin (1937, 1939) has shown, on the basis of 624 counts, that lead shot of uniform diameter, compressed to eliminate interstices, have an average number of faces also very close to fourteen (14.17). In the number of contacts, then, the compressed lead shot of uniform diameter and the cells of undifferentiated tissues show striking similarity. In addition to hexagonal and square faces, many of those of the lead shot were pentagonal.

The significance of the tetrakaidecahedron in the biological realm has been extended by Glaser and <sup>1</sup> Received for publication January 12, 1939. Child (1937) and by Glaser (1938); it has been considered also from other standpoints by Marvin (1936), Lewis (1937), and Matzke (1927, 1931).

MATERIAL AND METHODS .- The experiments described below were done with patent finish drop shot of two diameters, the "large" shot of approximately 2.54 millimeters (.10 inch), the "small" of approximately 1.27 millimeters (.05 inch). Before being used, each shot was examined under a binocular dissecting microscope magnifying 25 diameters, and those showing imperfections in shape, size, or surface were discarded. The large and small shot in varying proportions were then mixed as uniformly as possible and carefully poured into a steel cylinder having a 25 mm. bore. In each experiment they were compressed by means of a steel piston, from above, with a pressure of 40,000 pounds.2 This was sufficient to eliminate completely the spaces between the shot but permitted their subsequent separation without great difficulty. This pressure made it possible to transfer the compressed shot, in one mass, from the steel cylinder to a glass cylinder, with an inside diameter of 25 mm., fitted with a wooden piston. The shot could then be picked off one by one. The upper and outer several layers were discarded in each case, and the data presented below are based entirely on a study of the inner shot. Each compressed shot was examined under a binocular dissecting microscope magnifying 25 and 50 diameters, being handled by the manipulator constructed by Marvin and previously described (Marvin and Matzke, 1939).

Three compressions were made, using three different proportions of large and small shot. In the first, 5 cc. of the selected small shot and 20 cc. of the selected large shot were mixed, giving a volume ratio of 1 small: 4 large. By number there were approximately twice as many of the small balls as of the large ones. The ratio of small to large shot used in the second compression, by volume, was 1 small: 1 large, 13 cc. of each having been mixed together. There were about eight times as many of the small shot by number as of the large in this experiment. In the third compression the volume ratios were

<sup>2</sup> The compressions were made in the Research Laboratories of the Department of Civil Engineering of Columbia University by Mr. John N. Kenyon, and it is a pleasure to acknowledge his ever generous and willing cooperation.

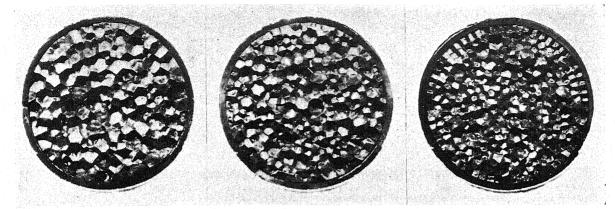


Fig. 1-3.—Fig. 1 (left). Surface view of cylinder of compressed lead shot used in experiment 1. Since the ratio of small to large shot, by volume, was 1:4, the large shot are more conspicuous than the small. The faces on many of the large shot are plainly evident. ×9/5.—Fig. 2 (center). Surface view of cylinder of compressed lead shot used in experiment 2. Equal volumes of small and large shot were used, and compressed shot of both sizes are clearly visible. ×9/5.—Fig. 3 (right). Surface view of cylinder of compressed lead shot used in experiment 3. The ratio of small to large shot, by volume, was 4:1, and consequently the small shot are much more conspicuous, though some of the large ones can also be seen. ×9/5.

4 small: 1 large, 20 cc. of the large and 5 cc. of the small having been used. By number in this third experiment there were approximately 32 small: 1 large.

DATA.—The general appearance of the shot in these three experiments is shown in figures 1, 2, and 3, which are photographs of experiments 1, 2, and 3, respectively. It is evident that the cylinder shown in figure 1 contains the largest proportion of the large shot, while that in figure 3 shows the greatest proportion of small shot. Figure 2 (experiment 2) is intermediate.

The number of faces on the compressed shot and the average number of faces is given in tables 1, 2, and 3. In the first experiment (table 1), in which

Table 1. Number of faces on compressed lead shot. Experiment 1. Ratio of amount of small to large shot by volume = 1:4. Ratio of diameters of small to large shot = 1:2.

Data on a	50 small	shot	Data on 50 large shot				
No. of faces per shot	No. of shot	Total faces	No. of faces per shot	No. of shot	Total faces		
6	1	6	15	1	15		
7	1	7	16	0	0		
8	7	56	17	2	34		
9	16	144	18	5	90		
10	17	170	19	12	228		
11	5	55	20	15	300		
12	2	24	21	6 6	126		
13	1	13	22	2	44		
			23	6	138		
	50	475	24	1	24		
				50	999		

Table 2. Number of faces on compressed lead shot. Experiment 2. Ratio of amount of small to large shot by volume = 1:1. Ratio of diameters of small to large shot = 1:2.

Data on	50 small	shot	Data on	Data on 50 large shot				
No. of faces per shot	No. of shot	Total faces	No. of faces per shot	No. of shot	Total faces			
9	3	27	19	2	38			
10	5	50	20	2	40			
11	7	77	21	1	21			
12	14	168	22	2	44			
13	15	195	23	3	69			
14	5	70	24	6	144			
15	1	15	25	6	150			
			26	8	208			
	50	602	27	5	135			
			28	7	196			
			29	4	116			
			30	4	120			
				50	1281			
Average no. shot = 602			Average shot =	no. of f 1281/50=				

the ratio of small to large shot was 1:4, by volume, the number of faces per small shot varied from 6 to 13, with the greatest frequencies at 9 and 10; the average number was 9.5. The data in all the experiments are based on 50 shot selected at random from the central portion of the cylinder. In the case of the 50 large shot tabulated for experiment 1, the number of faces per shot varied from 15 to 24, the greatest number having 19 and 20 faces, and the average being 19.98. Marvin (1937, 1939) found that the average number of faces per compressed shot, with all interstices eliminated, was approximately 14, if all the shot were uniform in size. He

used both the large and small shot here tabulated, and when either was used alone, the average number of faces was about 14. It may be concluded from the data here presented, then, that when the large and small shot are mixed in the proportions indicated, the number of faces on the small shot drops from 14 to about 9.5, while the number of faces on the large shot increases from 14 to approximately 20.

Table 3. Number of faces on compressed lead shot. Experiment 3. Ratio of amount of small to large shot by volume = 4:1. Ratio of diameters of small to large shot = 1:2.

Data on	50 small	shot	Data on	50 large	shot
No. of faces per shot	No. of shot	Total faces	No. of faces per shot	No. of shot	Total faces
10	1	10	25	2	50
11	3	33	26	2	52
12	8	96	27	2	54
13	17	221	28	8 1	224
14	12	168	29	6	174
15	7	105	30	6	180
16	2	32	31	11	341
		-	32	3	96
	50	665	33	5	165
			34	3	102
			35	1	35
			36	1	36
				50	1509
Average no. shot = 668				no. of f 1509/50	

In the second experiment, in which equal volumes of small and large shot were used (13 cc. of each), the number of faces on 50 small shot selected at random varied from 9 to 15, 12 and 13 being the commonest with the average at 12.04. The 50 large shot in this same cylinder showed a range in the number of contacts from 19 to 30, with an average of 25.62, and the greatest frequencies at 24, 25, 26, 27, and 28. In this case the large shot were more frequently in contact with small shot than in experiment 1, and hence the number of contacts on the large shot is greater. The small shot probably were also more frequently in contact with small shot than in experiment 1, since there were more of the small shot in experiment 2, and therefore the number of contacts came closer to 14 than in the first experiment.

In experiment 3 the ratio, by volume, of small to large shot was 4:1, 20 cc. of the small and 5 cc. of the large shot having been used. The ratio in number of shot was approximately 32 small:1 large. The number of faces on the 50 small shot examined varied in this experiment from 10 to 16, 13 and 14 being the most common, with the average at 13.3. In the 50 large shot chosen at random the number of contacts ranged from 25 to 36, with the greatest frequency at 31 and the average at 30.18. Under

the conditions of this experiment, small shot are nearly always in contact with small shot, and consequently the average (13.3) of the small shot comes close to 14, which would have been the average if only small shot had been used. The large shot has more numerous contacts in this experiment than in either of the others, presumably because under these conditions the large shot is surrounded mostly or in some cases perhaps entirely by small shot. If the diameters of the small and large shot are kept at 1:2, increase in the proportion of small to large shot, by volume, would not continue to increase the number of faces on the large shot indefinitely. The large shot would achieve the maximum number of contacts when completely surrounded by small shot. and further increase in the proportion of small shot would have no effect on the number of faces of the large shot. Under these conditions the number of faces on the small shot would come closer and closer

These results of experiments 1, 2, and 3 are shown graphically in figure 4, in which curve AA is based on the data for shot of uniform diameter (Marvin, 1939); BB represents the data of table 1 (experiment 1) on small shot, B'B' the data of table 1 (experiment 1) on large shot; CC and C'C' are curves for the data on the small and large shot, respectively, in table 2 (experiment 2), and DD and D'D' for the data on small and large shot, respectively, in table 3 (experiment 3). It is evident that curves BB, CC, and DD get progressively closer to AA, while curves B'B', C'C', and D'D' get progressively farther away from AA.

Table 4. Data on faces and angles on compressed lead shot. Experiment 1. Ratio of amount of small to large shot by volume = 1:4. Ratio of diameters of small to large shot = 1:2.

Data on 50 sm	nall shot	Data on 50 la	rge shot	
No. of edges per face	No. of faces	No. of edges per face	No. of faces	
3	22	3	23	
4	164	4	179	
5	217	5	380	
6	65	6	250	
7	7	7	140	
	-	8	22	
	475	9	5	
			999	
No. of tribed		No. of trihedral angles 1790		
No. of tetrahe	edral angles	No. of tetrahe	edral angles	

The data presented thus far deal with the number of faces on each of the compressed lead shot. In tables 4, 5, and 6 the kinds of faces, whether triangular, quadrilateral, pentagonal, etc., on the small and large shot in experiments 1, 2, and 3, respec-

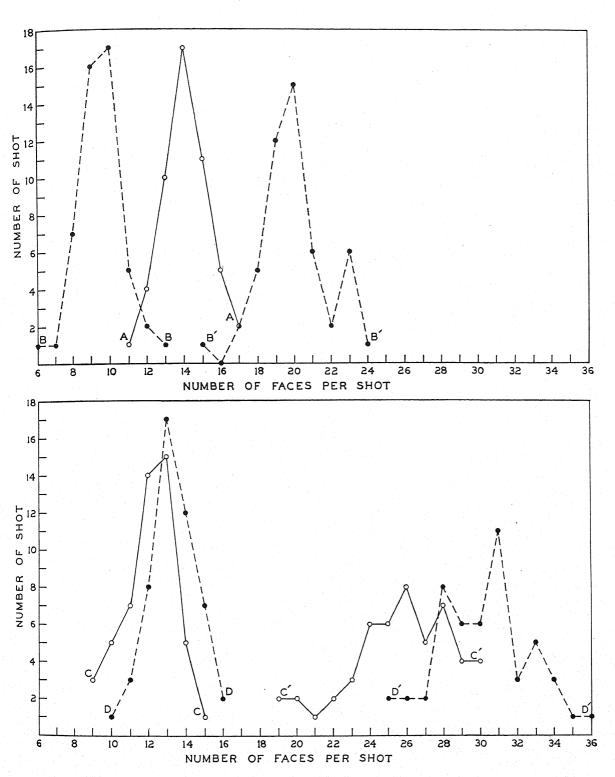


Fig. 4. Curves showing the number of faces per shot and the frequency of the occurrence of shot with these varying numbers of faces. AA is the curve for shot of uniform diameters (Marvin, 1939); BB and B'B' are the curves for the small and large shot, respectively, in experiment 1; CC and C'C' the curves for the small and large shot, respectively, in experiment 2; DD and D'D' the curves for the small and large shot, respectively, in experiment 3. BB, CC, and DD get successively closer to AA, while B'B', C'C', and D'D' get successively farther away.

Table 5. Data on faces and angles on compressed lead shot. Experiment 2. Ratio of amount of small to large shot by volume = 1:1. Ratio of diameters of small to large shot = 1:2.

Data on 50 small shot	Data on 50 la	rge shot	
No. of edges No. of per face faces	No. of edges per face	No. of faces	
3 27	3	26	
4 138	4	177	
5 280	5	465	
6 127	6	406	
7 27	7	154	
8	8	45	
	9	7	
602	10	1	
		1281	
No. of trihedral angles 1000	No. of trihedral angles 2334		
No. of tetrahedal angles	No. of tetrahe		

Table 6. Data on faces and angles on compressed lead shot. Experiment 3. Ratio of amount of small to large shot by volume = 4:1. Ratio of diameters of small to large shot = 1:2.

Data on 50 s	mall shot	Data on 50 large shot			
No. of edges per face	No. of faces	No. of edges per face	No. of faces		
3	29	3	52		
4	151	4	207		
5	263	5	437		
6	177	6	532		
7	40	7	201		
8	5	8	68		
		9	10		
	665	10	2		
			1509		
No. of tribe	· ·	No. of trihedral angles 2786			
No. of tetral	. •	No. of tetrahe			

tively, are given. From table 4, for instance, it is evident that in experiment 1 the 50 small shot had a total of 22 triangular faces, 164 quadrilateral faces, 217 pentagonal faces, etc. Similarly in the 50 large shot there were 23 triangular faces, 179 quadrilateral faces, 380 pentagonal faces, etc. These faces were sometimes plane, sometimes more or less curved. Similarly, the edges were straight lines in some cases, in others not.

The results in tables 4, 5, and 6 are presented graphically in figure 5, in which BB is the curve for the data on the small shot in table 4 (experiment 1), B'B' the curve for the data on the large shot in table 4 (experiment1). Similarly CC and C'C' are

the curves for the data on the small and large shot, respectively, in table 5 (experiment 2), while DD and D'D' are the curves for the data on the small and large shot, respectively, in table 6 (experiment 3).

From these tables and curves it is evident that there were more pentagonal faces than any other kinds in the small shot in each of the three experiments. Of the total of 1742 faces on the 150 small shot, 760 were pentagonal. While the slope of the three curves, BB, CC, and DD is in general similar, both the number and proportion of hexagonal faces is least in experiment 1 (BB) and greatest in experiment 3 (DD).

An examination of the data and curves dealing with the large shot in each of these three experiments as given in tables 4, 5, and 6 and curves B'B'. C'C', and D'D' in figure 5 reveals that pentagonal faces were more common than any other kind in experiments 1 and 2, but that hexagonal faces were of greatest frequency in experiment 3 (D'D'). It is obvious that there were many more pentagonal than hexagonal faces in the large shot in experiment 1. and many more hexagonal than pentagonal faces in the large shot in experiment 3, while the large shot in experiment 2 were intermediate in this respect. The proportion of pentagonal to hexagonal faces changes very definitely in the large shot in these three experiments. The same is true to a less extent in the small shot.

Tetrahedral angles, in which four faces on a compressed shot meet in a point, were recorded in each of the three experiments. They were of occasional occurrence in the small shot and more frequent in the large shot. In the large shot they were least common in experiment 1, which had the fewest and consequently the largest faces, and most abundant in the large shot in experiment 3, which had the most numerous and consequently the smallest faces. In a liquid system tetrahedral angles are unstable (Kelvin, 1887). This can hardly be true in the same sense in the experiments under consideration here. In a system of solids, tetrahedral angles should be more and more common as the faces get smaller, since the likelihood of four faces meeting in a point becomes greater as the area of the individual faces becomes reduced. This was the case in the experiments just described. It was somewhat difficult to decide, in certain cases, whether a tetrahedral angle had actually occurred or not. All such angles were studied under a magnification of 50 diameters, and if they still appeared to have four faces meeting in a point, they were designated as tetrahedral. There were in all 39 tetrahedral angles and 9784 trihedral angles on the 300 shot examined, as well as 14,754 dihedral angles. The ratio of tetrahedral to trihedral angles was therefore 1:251. Relatively very few tetrahedral angles were present, in spite of the fact that surface tension can hardly be very significant in this system.

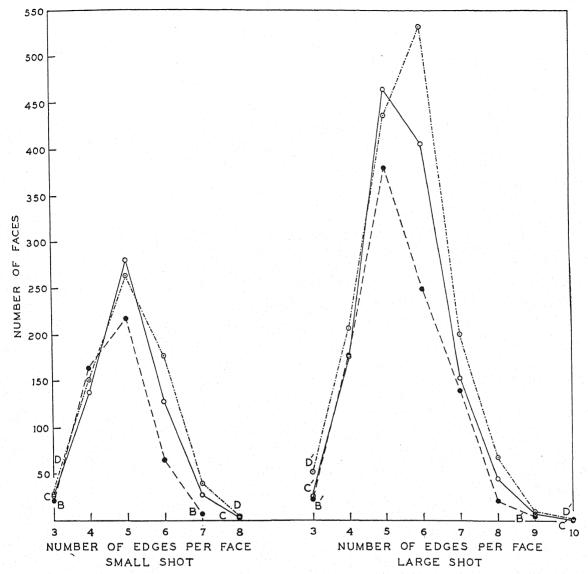


Fig. 5. Number of edges per face and the frequency of their occurrence (3-triangular, 4-quadrilateral, 5-pentagonal, etc.) in small and large shot. BB and B'B' are the curves for the small and large shot, respectively, in experiment 1; CC and C'C' the curves for the small and large shot, respectively, in experiment 2; DD and D'D' the curves for the small and large shot, respectively, in experiment 3.

It seems scarcely necessary to tabulate here the complete data on each of the 300 compressed lead balls studied, since the results have been presented in summary form above. Of the 300 shot carefully examined, one small one, in experiment 1, had only six faces, and these were all four-sided. Only one other shot, a small one in experiment 3, had faces all of one kind, twelve faces, all pentagonal. This was a pentagonal dodecahedron, showing some inequality in the faces and edges. None of the other 298 shot had the faces all alike. Of the 150 small shot 18 had only quadrilateral and pentagonal faces, in varying numbers, while one small shot in experiment 3 had 6 quadrilateral and 5 hexagonal faces, and another in experiment 1 had 6 quadrilateral

and 4 hexagonal faces. Many of the small and a few of the large shot had three kinds of faces, usually quadrilateral, pentagonal, and hexagonal, and many had more than three kinds of faces. None of the shot studied had twelve quadrilateral faces, as in the rhombic dodecahedron, nor were there any with eight hexagonal and six quadrilateral faces as in the orthic tetrakaidecahedron, though the two mentioned above did have only hexagonal and quadrilateral faces. The pentagonal dodecahedron was early discarded as a possible shape for cells in favor of the rhombic dodecahedron, since the former will not stack without interstices. The latter is also no longer accepted.

DISCUSSION AND CONCLUSIONS.—The data presented above indicate that in a system composed essentially of solids, in which surface tension is reduced to a minimum, the number of contacts is closely correlated with the proportion of small and large spheres. The work of Marvin (1937, 1939) shows that in solid spheres of uniform size, compressed to eliminate interstices, the number of contacts is 14 (14.17). Lewis (1923, 1925, 1933) found that cells in undifferentiated tissues have an average of almost exactly 14 faces (13.97 for 250 cells), so that there is a striking parallelism between the shot and the cells. It is to be expected, then, that there may well be correlations between large and small shot compressed together and large and small cells in tissues. The diameter ratios used in the shot, 1:2, may be expected of cells in tissues, and the ratios of the shot to the diameter of the cylinder are also in the same order of magnitude as the ratio of cell to pith diameters in the stems of certain plants.

When small and large shot are mixed together in the same cylinder and compressed, the number of faces on the small balls drops below 14, the number of faces on the large ones rises above 14. Using diameter ratios of 1:2, the average number of contacts of the small shot dropped to 9.5 when there was a large proportion, by volume, of the large shot; it rose to almost 14 (13.3) when a large proportion, by volume, of small shot was utilized. The greater the proportion of small shot, the closer the approximation was to 14. In the case of the large shot, a high proportion of this with reference to small shot caused an increase in the number of contacts from 14 to 19.98. As less and less of the large shot was mixed with the small, the number of faces on the large shot increased—to an average of 30.18 in experiment 3. Large shot surrounded by large shot have 14 faces on compression, but large shot partially or completely surrounded by small shot have more than fourteen. Under these varying conditions it is not possible to conceive of a polyhedron with a definite number of sides as an ideal type. The number of contacts in these experiments depends on the proportion of small and large shot; it is relatively fixed only for any one set of fixed conditions. In as far as similar factors hold for cells in tissues, the average number of contacts may be expected to vary with the proportion of small and large cells. If the cells are relatively uniform in size and intercellular spaces are insignificant, the number of faces expected is 14, as has been found to be the case (Lewis, 1923). If diameter ratios of the cells vary, the smaller cells would be expected to have fewer than 14 faces, the larger cells more than 14.

In the experiments described above, the diameter ratio of 1:2 was kept constant. Changing this ratio would also have an effect on the number of contacts. If a ratio of 1:4 were used, for instance, a still greater number of faces would be expected on the large shot. Under varying conditions of diameters

and number or volume of shot no one ideal type with a fixed number of faces holding for all experiments would be expected. It did not occur in the work described.

As the number of contacts changes with the varying proportions of small and large shot, so the proportion of pentagonal and hexagonal faces also varies under these changing conditions. This is especially evident from the large shot in experiments 1, 2, and 3. In the large shot in experiment 1 pentagonal faces were by far the most common, in experiment 3 there was a marked preponderance of hexagonal faces. An ideal type of contact or a fixed proportion of quadrilateral, pentagonal, hexagonal. etc., faces, rigid under the varying conditions in these experiments, is therefore also inconceivable. The number of contacts changes with the varying proportions of large and small shot, and the relative abundance of the kinds of contacts, whether triangular, quadrilateral, etc., similarly changes.

In experiment 1, in which there were numerically approximately 2 small shot to 1 large, the average number of faces, considering small and large shot together, was 13.0. In experiment 2, in which about 8 times as many of the small as of the large shot were used, the average of all the central shot rose to 13.6; in experiment 3, with 32 small to 1 large shot, the average was 13.8. Professor Lewis has kindly suggested that "it has previously been shown that in an aggregation of 14-hedral cells, if divisions occur in hexagonal planes (avoiding the unstable tetrahedral angles or edges where four cells meet), the average of the 14 facets will be maintained. It will rise if the division planes have more than 6 sides; it will fall if these planes are pentagonal, quadrilateral, or triangular. Thus averages of 13 sides per cell, as found in these shot, might be produced in organic tissue by division, as well as by

adjustment in proportion to size."

It is true that lead shot and cells in tissues differ greatly in many respects. Presumably the apical meristems in stems and roots as well as the regions of differentiation contain cells with liquid or semiliquid contents in which surface tension phenomena, colloidal properties, and biological factors are operative, none of which play any important part in lead shot. On the other hand, contact and pressure are of significance also in determining the shape of cells, as they are in the lead shot—more so, undoubtedly, in certain regions and tissues of the plant than in others. Although there are differences, this similarity is an important one, especially in view of the close correlation established by Lewis and Marvin between cell shapes and compressed shot of uniform diameters. The movement of the shot in one direction in the cylinder may be looked upon as one of the shortcomings of the experiments and is in no way correlated with the much-discussed question of slipping and gliding growth.

The data presented above throw light on one set of factors, contact and pressure, in cell shape determination. The mechanics of the process cannot be fully understood until the other factors have also been studied. The results here given will take on added significance when cells of varying sizes in the same tissue have been modeled and their three-dimensional shape has been determined. The evidence from the lead shot indicates that such cells will vary in the number of contacts, and consequently in shape, depending, partly at least, on the relative sizes and numbers of large and small cells; it shows also that the abundance of triangular, quadrilateral, hexagonal, etc., faces will be affected by the proportion of the cells of each size that occur in the tissue.

#### SUMMARY

Previous work of Lewis shows that cells in undifferentiated tissues have an average of 14 faces, while that of Marvin proves that in lead shot of uniform diameters compressed so that there are no interstices, there are also approximately 14 contacts

Since cells in tissues vary in size, lead shot of two diameters, with a ratio of 1:2, i.e., with volumes of

1:8, were mixed in varying proportions and compressed to eliminate all spaces.

Under these conditions the average number of faces on the small shot was always less than 14, varying from 9.5 to 13.3, while the average number of contacts on the large shot was always greater than 14, ranging from 19.98 to 30.18.

The average number of faces of all the central shot, large and small, considered together, varied from 13.0 to 13.8, giving fairly close approximations to the results obtained by Lewis in cells.

The proportion of hexagonal and pentagonal faces, especially on the large shot, varied, depending upon the conditions of the experiments.

In as far as contact and pressure determine cell shapes, it is to be expected that the association of small and large cells in the same undifferentiated tissue will result in fewer than 14 faces on the smaller cells and in more than 14 contacts on the larger ones.

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# THE USE OF INDICATOR PLANTS IN LOCATING SELENIFEROUS AREAS IN WESTERN UNITED STATES. II. CORRELATION STUDIES BY STATES 1

O. A. Beath, C. S. Gilbert, and H. F. Eppson

The basic purpose of our correlation of seleniferous plants vs. rocks has been to determine to what extent certain native plants can be depended upon to indicate a seleniferous soil where environmental factors are known to be different from those in Wyoming. In those states in the Rocky Mountain region having geological formations stratigraphically equivalent in age (e.g., Cretaceous and Tertiary) to those of Wyoming, it was expected that a certain degree of similarity would prevail as to the selenium content of the rocks and the indicator plants rooted in them.

Byers (6) (1935 report) stated, "It was hoped that certain plants might serve as indicators either by their presence or absence or that contours or other soil conformities might aid. No indications based upon plant distribution were detected by Mr. Piemeisel nor did any topographical indications seem useful." In 1936 the authors undertook to study the character of seleniferous indicator plants outside of Wyoming. The data in this paper include selenium analyses of indicator plants and a certain amount of miscellaneous vegetation from Arizona, California, Colorado, Idaho, Montana, Nevada, New Mexico, North Dakota, Oregon, South Dakota, Texas, and Utah. Seleniferous indicator plants from these states are correlative with geological formations comparable in age to those previously described by the authors, and in addition to those we have introduced several formations believed to be new assignments.

Data on the seleniferous vegetation of Wyoming have been rather fully discussed in earlier reports (1, 2, 3, 5, 12). The tabulation of rock and soil analyses (selenium) have been purposely omitted from this treatise. Unless samples of this character are taken so as to be representative of the seleniferous areas in which a plant's roots are imbedded, misleading results may be obtained. Furthermore the total amount of naturally occurring selenium is no index as to its availability (3, 12), nor is it always certain to indicate the probable seleniferous status of a natural selenium-absorbing plant.

Confirmatory evidence shows that a native indicator plant can become extremely seleniferous on a soil carrying moderate concentrations. On the other hand, the same plant species may be only moderately seleniferous on a soil carrying high concentrations of selenium. Analytical data involving the selenium content of rocks and soils are important in interpreting the broad aspects of the selenium problem.

Vegetation influenced by its selenium content is primarily a range problem. This is due to the fact that the natural habitat of the main absorbing plant

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species remains quite undisturbed. Consequently the authors have confined their investigations largely to those phases that apply to the grazing sections. We have not been concerned with isolated occurrences of selenium in ore bodies or specially enriched zones incapable of supporting a significant areal seleniferous plant cover.

Marine sediment relationships.—Geological formations stratigraphically equivalent in age vary in character and in thickness from one area to another in Wyoming. Likewise similar variations occur in the stratigraphy of the Rocky Mountain region.

Marine sedimentary rocks which were deposited contemporaneously are subject to striking lateral changes in their lithology, or physical constitution. Sandstones deposited adjacent to ancient shores grade into mudstones which were deposited farther from shore. These, in turn, grade into limestones deposited far from shore over areas in which the deposition of land-derived fragmental material was at a minimum. It is thus apparent that different kinds of sediments are deposited over adjacent areas at the same time, and the deposits are therefore said to be of the same age. Each of these rock types, of the same age but of different lithology, is referred to as a facies.

Contemporaniety of facies is determined through the use of fossils. It is believed that a species appeared, flourished for a certain length of time, then became extinct. Consequently, when specimens of species whose time-span is known to have been brief are found in different localities, the beds in which they are found, irrespective of lithology, are thought to have been deposited during the same geologic time.

Good examples of lateral variation and gradation (fig. 15) are found in the Cretaceous rocks of the Great Plains and the Rocky Mountains. Fossils

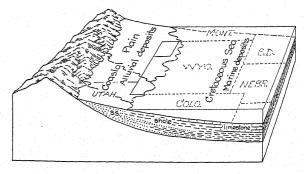


Fig. 15. Diagram of the central portion of the Rocky Mountain Region showing the contemporaneous deposition of marine and non-marine sediments during early-Upper Cretaceous time.

which characterize Niobrara time (the time during which the Niobrara formation was deposited) are found in the Fort Hays limestone and the Smoky Hill chalk of central Kansas (19), in the Niobrara calcareous shale in the Rawlins uplift of south central Wyoming (8), and in the sandstones of the upper Frontier formation of southwestern Wyoming (20). It is believed, therefore, that the limestone, shale, and sandstone represent contemporaneous deposition at different distances from the land mass to the west of Wyoming which bordered the Cretaceous sea. With such a manifest change in sediments in the Niobrara formation over areal distribution of several hundred miles, it is not surprising to find variations in the selenium content of the rocks of this type. In general the Hilliard and Cody shales of western Wyoming are much leaner in selenium than the calcareous shales of central Wyoming. This relationship is borne out by a comparison of the seleniferous condition of the same plant species from these

A similar gradation in lithology is shown by somewhat older Cretaceous rocks across the same area. A widespread fauna, characterized by certain fossil cephalopods, occurs throughout a large area in the Great Plains and Rocky Mountains. These fossils are found in the Codell sandstone member of the Carlile shale in Kansas and eastern Colorado, and in the Wall Creek sandstone member of the Frontier formation in south central Wyoming. In central Kansas, the Codell lies about 200 feet stratigraphically above the top of the Fairport chalky member of the Carlile, about 85 feet thick, which rests on the Greenhorn limestone, about 100 feet thick. Therefore, some 300 feet of calcareous beds lie 200 feet below the Codell sandstone. In south central Wyoming the Wall Creek sandstone rests on a noncalcareous shale sequence nearly 1000 feet thick. Consequently, at the time that calcareous sediments were being deposited in Kansas, muds were being deposited in south central Wyoming. In addition, some of the coal-bearing continental standstones in the lower Frontier of southwestern Wyoming were deposited at essentially the same time.

The illustrations just cited show in principle what may be expected to apply rather generally to all the sediments deposited during Cretaceous time. Formations of continental origin, of course, are made up largely of the source rocks from which the sediments were derived.

The subject matter and experimental data that follow are presented separately for each state. The investigation of seleniferous indicator plants and their geological relationships involving several western states was initiated in 1936. Consequently the data include material obtained over a period of three years. Collecting samples from all these areas and the inclusion of pertinent range data were possible only through the cooperation of several agencies. In this we were aided by representatives of the U. S. D. A. Soil Conservation Service, U. S. Forest

Service, U. S. Biological Survey, botanists, geologists, archaelogists, chemists, and others.

It is our hope that the data given will be interpreted within the limitations previously discussed in this report.

In presenting the results of analysis of plants from various states, the following information is listed for each sample: name; laboratory sample number; part of plant analyzed; stage of growth when plant was collected; location; geological data consisting of (1) type of rock on which the plant was growing, (2) geological formation or group to which the rock type belongs, and (3) the geological age to which the formation or group is assigned; and finally, the selenium content of the plant expressed in parts per million (p.p.m.). In some instances not sufficient geological data are as yet known to us to give all the desired information, in which case a dash indicates the incompleteness of the geology.

The selenium content of the plants is reported on a bone dry (b.d.), air dry (a.d.), or partly dry (p.d.) basis. Where the result is given as negative (neg.), there was either no selenium found by analysis or the amount present was less than 1 p.p.m. on a bone dry basis, an amount deemed insignificant from an economic viewpoint.

# ARIZONA

The principal indicator plants occupying seleniferous areas in Arizona are species of Stanleya and Astragalus. It will be observed that a large number of species of Astragalus were collected and analyzed for selenium. These analyses were made to determine which species of Astragalus are indicators of a seleniferous soil. It is apparent from these analyses that indicator plants are restricted to limited areas. This point is clearly illustrated by the comparatively wide distribution of the non-selenium-bearing Astragalus diphysus, A. wootoni, A. calycosus, and the restricted distribution of the selenium-absorbing Astragalus praelongus or A. confertiflorus.

There are several areas in Arizona where Stanleya pinnata, Astragalus praelongus, and A. preussii are reported to occur that have not been investigated. The data given in this report include analyses of vegetation associated with representative rock types from a number of places. The most seleniferous plants were found to occur in northeastern Arizona. This conclusion is based upon the evidence supplied by analyses of Astragalus praelongus, indigenous to the Winslow-Holbrook section. Rocks in this area that are correlative with seleniferous plants are Triassic (basal) in age and are equivalent stratigraphically to the basal Chugwater (5) formation (Triassic) of Wyoming and Colorado.

In the Kaibab Reservation the seleniferous Astragalus confertifiorus was collected on what is believed to be the Moenkopi formation. The Moenkopi is Triassic (basal) in age. Reference has been made to this formation in connection with certain selenif-

erous vegetation in southern Utah. Consequently its seleniferous character seems to be definitely established in the areas studied.

Stanleya pinnata occurs in the Tonto basin of central Arizona. It grows in scattered patches from the Payson area to the Roosevelt Reservoir. Composite samples, at the early flower stage of growth, were selected from several areas in the valley. Selenium was present in all the samples. The sediments in which the Stanleyas were rooted are believed to be Pliocene (Tertiary) (22) in age. Reference has been made to the probable origin of analogous seleniferous rocks elsewhere—e.g., the Payette formation of southwestern Idaho—placing emphasis upon the fact that the older rocks from which these seleniferous sediments were derived is particularly significant.

The seleniferous Astragalus sabulosus has been observed to occur in the Upper Verde Valley of north central Arizona. In this locality the valley floor sediments (Verde formation), in which the A. sabulosus grows, are Tertiary or Quaternary (9) in

A sample of Stanleya pinnata from the Soil Conservation Service nursery near Tucson was seleniferous. The specimens were growing in a soil derived from rocks of a local character commonly referred to as caliche.

Stanleya pinnata was observed to occur along the Bright Angel Trail in the Grand Canyon. It was not found to occur until the Trail intercepted the Supai formation a considerable distance below the south rim of the canyon. The Supai is of Permian and Pennsylvanian age.

Aster abatus (Xylorrhiza tortifolius) is reported to occur in a number of areas in western Arizona. Type specimens from Utah and California were not seleniferous. It is believed that Aster abatus is not dependable as an indicator of a seleniferous soil.

# Arizona Plant Samples

- Astragalus species, E86; prebloom; tops; Duncan; Igneous, Tertiary; Neg.
- A. species, D74; tops; prebloom; near Fossil Forest; Shale, Moenkopi Fm.?, Triassic; 1 p.p.m., b.d.
- 1. species, E651; tops; seed; Bear Flats Junction, 8 mi. N. of Young; Igneous, —, Cretaceous?; Neg.
- A. species, E652; tops; seed; Heber; Shale, Kaibab Fm.?, Permian; Neg.
- Astragalus calycosus Torr., E273; tops; past bloom; Vermilion Cliffs; Alluvium, —, Triassic; Neg.
- A. calycosus Torr., E303; tops; past bloom; Grand Canyon; South Rim; Limestone, Supai Fm., Perm. & Penn.; Neg.
- A. calycosus Torr., E408; tops; past bloom; Seligman; Massive limestone, Supai Fm.?, Perm. & Penn.; Neg.
- A. confertiflorus Gray, E269; tops; past bloom; Kaibab Reservation; Shale, Moenkopi Fm.?, Triassic; 27 p.p.m., b.d.
- A. diphysus Gray, E275; tops; past bloom; Kaibab Reservation; Shale, Moenkopi Fm.?, Triassic; Neg.
- A. douglasii (T. & G.) Gray, E146; tops; bloom; 10 mi.
   S. of Salt River Crossing; —, Apache Group, pre-Cambrian; Neg.

- A. flexuosus Dougl., E144; tops; bloom; 10 mi. S. of Salt River Crossing; —, Apache Group, pre-Cambrian; Neg.
- A. flexuosus Dougl. & A. humistratus Gray, E115; tops; in bloom; Pina Blanca; Igneous, —, Cret. & Tert., Neg.
- A. humistratus Gray, E274; tops; past bloom; Vermilion Cliffs; Alluvium, —, Triassic; Neg.
- A. layneae Greene, E277; tops; past bloom; Near Kingman; Alluvium, —, Quaternary?; Neg.
- A. leucolobus Jones, E270; tops; past bloom; Vermilion Cliffs; Alluvium, —, Triassic; Neg.
- A. nitidus Dougl., E141; tops; in bloom; St. John's; Shale, Chinle Fm., Triassic; Neg.
- A. (Hamosa) nothoxys Gray, E114; tops; in bloom; Sycamore Canyon, S. of Ruby; Igneous, —, Cret. & Tert.; Neg.
- A. nuttallianus DC., E271; tops; past bloom; Cedar Ridge; Shale, Chinle Fm.?, Triassic; Neg.
- A. palans Jones, C144; flowers & seeds; seed; 30 mi. N. of Cameron; Shale, Chinle Fm., Triassic; Neg.
- A. praelongus Sheld., D75; tops; prebloom; Fossil Forest; Shale, —, Triassic; 4500 p.p.m., b.d.
- praelongus Sheld., D76; tops; prebloom; 10 mi. W. of Winslow; Shale, —, Triassic; 3380 p.p.m., b.d.
- A. puniceus Osterh., E272; tops; past bloom; Cedar Ridge; Shale, Chinle Fm.?, Triassic; Neg.
- A. sabulosus Jones, F46; tops; prebloom; Herb. spec.; Camp Verde; Alluvium, Verde Fm., Tert. or Quat.; 1734 p.p.m., a.d.
- A. wootoni Sheld., E145; tops; bloom; 10 mi. S. of Salt River Crossing; —, Apache Group, pre-Cambrian; Neg.
- A. wootoni Sheld. & A. lentiginosus Dougl., E100; tops; in bloom; San Simon; Alluvium, —, Quaternary?; Neg.
- Atriplex species, C145; flowers & seeds; seed; 13-17 mi. N. of Cameron; Shale, Chinle Fm.?, Triassic; Neg.
- Mentzelia species, E87; leaves & stems; in bloom; near Duncan; Igneous, —, Cret. & Tert.; Neg.
- M. species, E89; tops; in bloom; 2 mi. S. of Globe; Conglomerate, Gila, Quaternary; Neg.
- M. species, E93; tops; prebloom; Tonto Basin; Shale,—, Pliocene; Neg.
- M. species, E99; tops; in bloom; Tucson; Caliche, —, Quaternary?; 10 p.p.m., a.d.
- Mustard species, E88; tops; in bloom; between Duncan & Safford; Igneous, —, Cret. & Tert.; Neg.
- Simmondsia californica Nutt., E95; leaves; in bloom; Tonto Basin; Shale, —, Pliocene; Neg.
- Stanleya species, E252; leaves & flowers; in bloom; Painted Desert; Shale, —, Triassic; 3 p.p.m., p.d.
- S. species, E545; leaves & seeds; past bloom; Vermilion Cliffs; Alluvium, —, Triassic; 1 p.p.m., b.d.
- S. pinnata (Pursh) Britt., D868; leaves & stems; past bloom; Tonto Basin; Alluvium, —, Pliocene; 59 p.p.m., a.d.
- S. pinnata (Pursh) Britt., E90; leaves & flowers; in bloom; Tonto Basin; Alluvium, —, Pliocene; 15 p.p.m., a.d.
- S. pinnata (Pursh) Britt., E91; leaves & flowers; in bloom; Tonto Basin; Alluvium, —, Pliocene; 23 p.p.m., a.d.
- S. pinnata (Pursh) Britt., E92; leaves & flowers; in bloom; Tonto Basin; Alluvium, —, Pliocene; 46 p.p.m., a.d.
- S. pinnata (Pursh) Britt., E142; leaves & flowers; in bloom; Tucson; Caliche, —, Quaternary?; 33 p.p.m.,

#### CALIFORNIA

The preliminary survey of the seleniferous plants in California was confined to the southern part, principally in Riverside, Imperial, and San Bernardino counties. Only those areas were selected that carried species of Stanleya, Astragalus limatus Sheld., and Aster abatus Greene (Xylorrhiza tortifolius Gray). In so far as our investigations have gone, it is apparent that the seleniferous Stanleya pinnata is associated with two geological formations, one the White Tank Monzonite and the other a poorly defined calcareous type of rock designated as caliche. The latter is much more common than the White Tank Monzonite in the areas studied. The only formation in the Twenty-nine Palms area capable of supporting Stanleya is the White Tank Monzonite. This formation is intruded into pre-Cambrian rocks and is believed to be of Jurassic age (15). Careful search failed to reveal Stanleya growing on any of the pre-Cambrian rocks exposed in this area. Areas in the Mohave Desert capable of supporting Stanleya pinnata appear to be confined to a rock type of a calcareous nature.

Aster abatus was found to occur on various rock types. In no sense is this species an indicator of a

seleniferous soil.

Astragalus limatus occurs abundantly in Imperial County near Truckhaven. It was selected for its selenium-absorbing properties because it belongs to the Preussii group. A. limatus was distinctly seleniferous in the areas investigated. It was found to

grow in sediments of Quaternary age.

The Stanleya elata occurs in areas in Death Valley. It is reported to be rather numerous in certain localities from 4000 to 5000 feet elevations. Mr. M. French Gilman, Death Valley National Monument, states that S. elata "grows on flats and canyons at these altitudes but does not seem to be very widely distributed over the valley at the proper altitudes." Mr. Gilman has also observed it growing in the Panamint and Grapevine Mountains. As a food, Mr. Gilman states, "The Indians use the young leaves as greens but tell me they have to throw out the first water when it boils and finish the cooking in fresh water, otherwise the greens make them sick if they do not cook it that way." Stanleya elata from the Death Valley has not been analyzed for selenium.

The selenium content of the Stanleyas included in our preliminary correlation studies is in general very low. There are two points to be considered in this connection. If selenium is essential to the normal development of the Stanleya species, how much of it is necessary? The other consideration has to do with the amount of selenium that would be necessary to cause a Stanleya to be toxic to physiological processes. The first query cannot be answered. The presumption might be that under extremely arid conditions a part or two per million of selenium in the plant is all that is necessary for the Stanleyas to develop normally. To be toxic a considerably larger concentration would be necessary. Just what

this would be cannot be given at this time. Our data are not complete for the seasonal variations of the selenium content in the Stanleyas investigated. In the Rocky Mountain region the Stanleyas start their growth directly from the roots. Stanleya pinnata in southern California sends out new leaves from the old stems. In the former, one would be concerned with a new development, while in the latter, the sampling would include partially dried out stems as well as green leaves. This disparity might make a considerable difference in the final analytical results.

The Stanleya collections, it will be observed, represent but one sampling. Some analyses were made from herbarium specimens and results reported merely as positive. While purely qualitative, it was concluded that an exact determination would in no way be representative of fresh material, hence the test was carried only to the point where selenium was definitely indicated.

A few soil and rock samples were collected and analyzed for selenium. Most of the material of this character was taken from the surface, consequently the results could not be considered representative of the seleniferous rocks where the *Stanleyas* were taken. These results and others will be held in abeyance until additional field evidence has been obtained.

The restricted growth of Stanleya pinnata in the areas studied indicates that it is extremely selective in its growth. Until evidence to the contrary is produced, its occurrence may be regarded as indicative of a seleniferous soil, at least sufficient so to supply the plant's requirement.

# California Plant Samples

Aster abatus Blake, E65; old stems; very young; T 1S, R 7E, SBM; San Bernardino Co. Palms Granite, —, pre-Cambrian; Neg.

A. abatus Blake, E66; crown; very young; about ½ mi. E. of Coyote Holes T 1S, R 7E; Palms Granite, pre-

Cambrian; Neg.

A. abatus Blake, E104; tops; bloom; 29 Palms T 1N, R 7E, S 11 SBM; Granite, pre-Cambrian; Neg.

A. abatus Blake, E148; tops; bloom; Rio Grande; Caliche,
—, Quaternary; Neg.

A. abatus Blake, E149; tops; bloom; Kramer; Caliche,
 —, Quaternary; Neg.

A. abatus Blake, E155; tops; bloom; West Pinto Basin, 31 mi. S. of Twenty-nine Palms; Palms Granite, —, pre-Cambrian; Neg.

A. abatus Blake, E157; tops; bloom; 1 mi. N. of Cottonwood Springs; Palms Granite, —, pre-Cambrian; Neg.

A. abatus Blake, E234; tops; bloom; Cushenbury Springs, Sec. 11, T 3N, R 1E; Alluvium, —, Quaternary; Neg.

A. abatus Blake, E265; tops; bloom; Sec. 31, T 1S, R 9E, SBM, same as E264; \*Monzonite, ——, Jurassic?; Neg. Astragalus species, E109; tops; bloom; between Baker &

Las Vegas; —, —, —; Neg.

Astragalus coulteri Benth., E143; tops; bloom; Midway; \_\_\_\_, \_\_\_; Neg.

\* White Tank Monzonite.

- A. limatus Sheld., F75; tops; seed; Herb. Spec.; Truck-haven; Alluvium; —, Quaternary; 183 p.p.m., a.d.
- A. limatus Sheld., F99; tops; early seed; Truckhaven; Alluvium; —, Quaternary; 614 p.p.m., b.d.
- A. malacus Gray, E105; tops; bloom; Twenty-nine Palms; Alluvium, —, Quaternary; Neg.
- Possibly Schoenocrambe, E108; leaves; young; near Rich's Well T 2N, R 5E, Sec. 12; Caliche, —, Quaternary; 2 p.p.m., b.d.
- Sphaeralcea ambigua Gray, E158; leaves; bloom; 1 mi. N. of Cottonwood Springs; \*Monzonite, —, Jurassic ?;
- Stanleya pinnata (Pursh) Britt., E2; tops; seed, Herb. spec.; Dos Palmos Springs; Caliche, —, Quaternary; Pos.
- S. pinnata (Pursh) Britt., E3; tops; seed, Herb. spec.; Lucerne Valley; Caliche, —, Quaternary; Pos.
- S. pinnata (Pursh) Britt., E45; leaves & old stems; prebloom; Twenty-nine Palms (15 mi. S. W.), T 1S, R 7E; \*Monzonite, ——, Jurassic?; Pos.
- S. pinnata (Pursh) Britt., E57; leaves; prebloom; Cottonwood Springs, T 5S, R 11E, SBM; \*Monzonite, ——, Jurassic?; 25 p.p.m., a.d.
- S. pinnata (Pursh) Britt., E106; tops; prebloom; Twentynine Palms; \*Monzonite, —, Jurassic?; 1 p.p.m., a.d.
- S. pinnata (Pursh) Britt., E147; tops; prebloom; Adelanto; Caliche, —, Quaternary; 2 p.p.m., a.d.
- S. pinnata (Pursh) Britt., E156; tops; prebloom; 1 mi. N. of Cottonwood Springs; \*Monzonite, —, Jurassic?; 8p.p.m., b.d.
- S. pinnata (Pursh) Britt., E233; tops; bloom; Lucerne Valley; Caliche, —, Quaternary; 46 p.p.m., b.d.
- S. pinnata (Pursh) Britt., E264; tops; bloom; S 31, T 1S, R 9E, SBM; \*Monzonite, ——, Jurassic?; 2 p.p.m., b.d.
- S. pinnata (Pursh) Britt., E287; tops; bloom; Cushenbury Springs, Sec. 1-2, T 3N, R 1E, SBM; Alluvium,
   —, Quaternary; 15 p.p.m., b.d.
- S. pinnata (Pursh) Britt., E113; leaves; young; Twenty-nine Palms; \*Monzonite, —, Jurassic?; 3 p.p.m., a.d.

# COLORADO

In 1936 the authors cooperated with the United States Soil Conservation Service in Colorado in the study of the distribution of seleniferous indicator plants. The areas included in these exploratory surveys were located in east-central Colorado. The native plants selected for study were Astragalus bisulcatus, A. racemosus, A. pectinatus, species of Oonopsis, Stanleya, and Xylorrhiza. The initial collections were confined to those sections where many of these indicator plants were reported to occur. Geological correlations were made after the chemical analyses had been completed. This special use of indicator plants is an excellent illustration of their value to range botanists in locating seleniferous soils particularly where the soil cover is not easily referable to a geological background. Reference to the chemical analyses in this report indicates the general consistency of the seleniferous character of the indicators collected by staff members of the Soil Conservation Service. Plants obtained by the survey are identified by the prefix-Colo. 1, 2 -Allred A, B, etc. The analyses also include mis-

\* White Tank Monzonite.

cellaneous samples of vegetation common to the areas explored. These analyses were made for comparative purposes. In the selection of grass samples it was the usual practice to obtain them from soils where the seleniferous indicator plants had had an opportunity to exert a favorable influence in the development of available selenium.

Preliminary studies of selenium occurrences in native plants in the Durango section were undertaken in 1937. These investigations were executed cooperatively with the wildlife division of the United State Forest Service. The important indicator plants were found to be Astragalus pattersonii, A. haydenianus, A. urceolatus, and A. confertiflorus. Astragalus pattersonii occurs widely distributed in the Durango area. It is restricted, as are all the other seleniferous Astragali, to definite geological formations. Mr. L. E. Coughlin, Wildlife specialist, also collected a number of the common forage plants and shrubs for selenium analysis. Its general absence from such vegetation is in agreement with results obtained elsewhere. Interpreted broadly, these results confirm the authors' conclusions that most forages and shrubs are incapable of absorbing selenium in toxic quantities from raw seleniferous shales.

In western Colorado our explorations brought us in contact with a number of seleniferous indicator plants. Collections were made usually from areas adjacent to the highways traversed. Consequently the extent to which the indicator plants are distributed geographically in this part of Colorado is not known to us. The indicator plants encountered were Astragalus confertiflorus, A. haydenianus, A. pattersonii, species of Xylorrhiza, and Stanleya. In the Craig-Meeker-Rifle area dense stands of Astragalus haydenianus and A. pattersonii were observed. These plants occupied pastures, roadside banks, and the borders of irrigated fields. Scattered patches of Stanleyas occurred in the same general areas.

In the Grand Junction-Fruita area are extensive growths of Stanleya, Xylorrhiza, and Astragalus. With the exception of the Xylorrhiza specimens, all the plants collected were green and vigorous. The stage of growth in which Xylorrhizas reach their maximum selenium intake has been considered (4).

The geological formations of Colorado, included in this report, that support seleniferous plants involve rocks of Jurassic, Cretaceous, and Tertiary age.

Morrison.—The Morrison formation is upper Jurassic (21) in age. Indicator plants growing on rocks of the Morrison in Colorado were seleniferous in the areas investigated. In its type section at Morrison, Colorado, the Morrison formation is 325 feet thick. Throughout Wyoming the Morrison formation ranges in thickness from 150 feet to 250 feet. The causes in thinning of this formation may be (1) deposition on an irregular erosional surface or (2) portions may have been removed by pre-Cloverly erosion. It is not known whether or not

the upper portion of the Morrison formation in its type locality is seleniferous. Scattered exposures of the Morrison in western Colorado have not been in-

vestigated by us for indicator plants.

Dakota Group.—Throughout much of Colorado and Wyoming there is a succession of standstones, shale, and conglomerates, for the most part of fresh water origin, which lie between the Morrison formation and the Benton shale. This succession has been referred to as the Dakota Group. It is believed to be of upper Cretaceous age. The basal portion may be older.

Cloverly formation.—In the Glendevey area of northern Colorado the basal conglomerate member of the Dakota group is referred to as the lower

member of the Cloverly formation.

Benton shale.—The Benton shale is upper Cretaceous in age. In parts of Wyoming and eastern Colorado the rocks (21) formerly called Benton are now divided into (descending) Carlile shale, Greenhorn limestone, and Granerous shale. In southcentral Wyoming and the Bighorn Mountains they are divided into (descending) Carlile shale, Frontier formation, Mowry shale, and Thermopolis shale.

Mancos shale.—The marine Mancos shale has yielded faunas (18) (in ascending order) of Greenhorn, Carlile, Niobrara, and Pierre age. It is upper

Cretaceous in age.

Niobrara formation.—The Niobrara is upper Cretaceous in age. It is the upper member of the Colorado group. The generally accepted (21) definition of the Colorado group includes only Benton and Niobrara and their equivalents.

Pierre formation.—The Pierre is upper Cretaceous in age. It is the lower formation of the Mon-

tana group.

Lewis formation.—The Lewis formation is upper Cretaceous in age. In a general way it is synchronous (17) with some part of the middle and upper portions of Pierre shale to the east of Rocky Mountains but more exact correlations cannot be made until further field evidence is available. The Lewis shale of southern Wyoming and northern Colorado is not (17) the same as the Lewis shale of San Juan Basin of southern Colorado. The Lewis shales of the San Juan Basin have not been explored for indicator plants.

Lance formation.—The age of Lance formation is in question. In northwestern Colorado it is prob-

ably upper Cretaceous in age.

Animas formation.—The Animas is probably Ter-

tiary (Eocene?) in age.

McDermott formation.—The McDermott forma-

tion is upper Cretaceous in age.

Wasatch Group (Wasatch formation).—The name Wasatch has been widely applied both as a group and as a formation to successions of rocks which are believed to have been deposited in Paleocene and lower Eocene through the Rocky Mountain region. These successions were deposited in the intermontane basins and the rock débris of which they are composed was derived from adjacent mountains;

consequently the succession varies widely from basin to basin. Locally (12) these rocks support seleniferous vegetation. In central and southern Wyoming the seleniferous indicator plants growing on these rocks are as seleniferous as those growing on rocks of Cretaceous age.

## Colorado Plant Samples

Agropyron riparium Scribn. & Smith, D 256; tops; seed; Durango; Shale, Mancos Fm., Cretaceous; 7 p.p.m., p.d. A. smithii Rydb., Colo. 18; tops; seed; near Scott; Shale, Pierre Fm., Cretaceous; 4 p.p.m., b.d.

A. smithii Rydb., Colo. 40; tops; flower; Jimmy Camp Creek; near Colorado Springs; Shale, Pierre Fm.,

Cretaceous; 4 p.p.m., b.d.

A. smithii Rydb., Colo. 55; tops; seed; Apache Creek near Lascar; Shale, Niobrara Fm., Cretaceous; 11 p.p.m., b.d.

Aristida longiseta Steud., Colo. 31; tops; flowers; 5 mi. E. of Colorado Springs; Shale, Lance Fm.?, Cretaceous; 20 p.p.m., b.d.

Asclepias galioides H.B.K., Colo. 15; top; bud; Hugo; Shale, Pierre Fm., Cretaceous; 10 p.p.m., a.d.

A. galioides H.B.K., D413; tops; flower; Cortez; Sandstone, Dakota Grp.?, Cretaceous; Neg.

A. galioides H.B.K., D334; tops; seed; Grand Junction; Shale, Mancos Fm., Cretaceous; Neg.

A. galioides H.B.K., D335; tops; seed; Loma; Shale, Morrison Fm., Upper Jurassic; 2 p.p.m., b.d.

A. galioides H.B.K., D480; tops; early seed; S. of Durango 11-12 mi.; Shale, Animas Fm., Eocene?; Neg.

A. galioides H.B.K., D560; tops; seed; 5 mi. N. of Loma; Shale, Mancos, Cretaceous; 11 p.p.m., a.d.

A. galioides H.B.K., T3; tops; seed; 7 mi. N.W. of Fruita; Shale, Mancos, Cretaceous; 4 p.p.m., a.d.

Aster species, Allred B; tops; flower; Ridgway; Shale, Morrison Fm., Upper Jurassic; Neg.

Aster glaucus T. & G., E507; tops; seeds; 20 mi. N. of Rifle; Shale, Wasatch Fm.?, Tertiary; 25 p.p.m., b.d.

Astragalus species, Allred 12; tops; seed; 5 mi. S. Pagosa Springs; Shale, Mancos Fm., Cretaceous; Neg.

A. species, Allred 1; tops; flower; 2 mi. N. of Kremmling; Shale, Niobrara Fm., Cretaceous; 313 p.p.m., a.d.

A. species, Colo. 43; tops; past seed; Jimmy Camp Creek, near Colorado Springs; Shale, Pierre Fm., Cretaceous; 19 p.p.m., b.d.

A. bisulcatus (Hook.) Gray, Allred 5; tops; seeds; Fremont County; Shale, Morrison Fm., Upper Jurassic;

183 p.p.m., a.d.

A. bisulcatus (Hook.) Gray, A39; tops; young; near Fort Collins; Shale, Morrison Fm., Upper Jurassic; Pos.

A. bisulcatus (Hook.) Gray, Colo. 4; tops; seed; Apache Creek near Lascar; Shale, Niobrara Fm., Cretaceous; 1620 p.p.m., b.d.

A. bisulcatus (Hook.) Gray, Colo. 10; tops; seed; Hugo; Shale, Pierre Fm., Cretaceous; 195 p.p.m., a.d.

A. bisulcatus (Hook.) Gray, Colo. 21; tops; seed; N. of Wellington; Shale, Pierre Fm., Cretaceous; 166 p.p.m., b.d.

A. bisulcatus (Hook.) Gray, Colo. 22; tops; seed; N. of Wellington; Shale, Pierre Fm., Cretaceous; 133 p.p.m., b.d.

A. bisulcatus (Hook.) Gray, Colo. 26; tops; seed; Trinidad; Shale, Pierre Fm., Cretaceous; 178 p.p.m., b.d.

A. bisulcatus (Hook.) Gray, Colo. 35; tops; seed; Jimmy Camp Creek near Colorado Springs; Shale, Pierre Fm., Cretaceous; 51 p.p.m., b.d.

- A. bisulcatus (Hook.) Gray, Colo. 41; tops; prebloom; Jimmy Camp Creek near Colorado Springs; Shale, Pierre Fm., Cretaceous; 98 p.p.m., b.d.
- A. bisulcatus (Hook.) Gray, Colo. 44; tops; seed; Jimmy Camp Creek near Colorado Springs; Shale, Pierre Fm., Cretaceous; 325 p.p.m., b.d.
- A. bisulcatus (Hook.) Gray, Colo. 45; tops; past seed; 1/8 mi. W. of Trinchera Creek near Trinchera; Shale, Benton Shale, Cretaceous; 19 p.p.m., b.d.
- A. bisulcatus (Hook.) Gray, Colo. 46; tops; past seed; 1/4 mi. W. Trinchera Creek near Trinchera; Shale, Benton Shale, Cretaceous; 125 p.p.m., b.d.
- A. bisulcatus (Hook.) Gray, Colo. 49; tops; seed; 10 mi.
   W. Trinchera Creek near Trinchera; Shale, Niobrara Fm., Cretaceous; 78 p.p.m., b.d.
- A. bisulcatus (Hook.) Gray, Colo. 54; tops; past seed; S. of Walsenburg; Shale, Pierre Fm., Cretaceous; 258 p.p.m., b.d.
- A. bisulcatus (Hook.) Gray, D85; tops; young; 5 mi. N. of Trinidad; Shale, Pierre Fm., Cretaceous; 1144 p.p.m., b.d.
- A. confertiflorus Gray, D211; flower & seed; Seed; Durango; Shale, Mancos Fm., Cretaceous; 160 p.p.m., b.d.
   A. confertiflorus Gray, D295; tops; seed; N.W. of Piedra;
- Shale, McDermott Grp., Cretaceous; 155 p.p.m., b.d. A. confertiflorus Gray, E246; tops; seed; Colo. Nat.
- A. confertifiorus Gray, £246; tops; seed; Colo. Nat. Monument near Grand Junction; Shale, Morrison Fm.?, Upper Jurassic; 312 p.p.m., b.d.
- A. confertiflorus Gray, T9; tops; seed; Grand Junction; Shale, Mancos Fm., Cretaceous; 888 p.p.m., a.d.
- A. drummondii Dougl., E1032; tops; flower, Herb. sp.; near Fort Collins; Shale, Morrison Fm., Upper Jurassic; Neg.
- A. goniatus Nutt., Colo. 25; tops; flower; near Como; Shale, Pierre Fm., Cretaceous; 6 p.p.m., b.d.
- A. haydenianus Gray, B424; tops; past seed; Steamboat Springs; Alluvium, ——, Cretaceous; 114 p.p.m., a.d.
- A. haydenianus Gray, D215; tops; seed; Pagosa Springs; Shale, Mancos Fm., Cretaceous; 25 p.p.m., b.d.
- A. haydenianus Gray, D233; tops; seed; Durango; Shale,
   Mancos Fm., Cretaceous; 39 p.p.m., b.d.
- A. haydenianus Gray, E488; tops; seed; 10 mi. S. of Kremmling; Shale, Pierre Fm.?, Cretaceous; 155 p.p.m., b.d.
- A. haydenianus Gray, T1; tops; seed; 24 mi. S. of Craig on rt. 13; Shale, Mancos Fm., Cretaceous; 2148 p.p.m., a.d.
- A. haydenianus Gray, T10; tops; seed; Meeker; Shale, Mancos Fm.?, Cretaceous; 27 p.p.m., a.d.
- A. haydenianus Gray, T11; tops; seed; 23 mi. N. of Rifle; Shale, Wasatch Fm.?, Tertiary; 175 p.p.m., a.d.
- A. haydenianus Gray, T12; tops; seed; Meeker; Shale, Mancos Fm., Cretaceous; 80 p.p.m., a.d.
- A. haydenianus Gray, T13; tops; seed; 2 mi. S. of Craig; Alluvium, Lewis Fm., Cretaceous; Pos.
- A. hylophilus (Rydb.) A. Nels., E419; tops; bloom; Bull Mt. N. of Glendevey; Conglomerate, Lower Cloverly Fm., Cretaceous; Neg.
- A. hylophilus (Rydb.) A. Nels., E422; tops; prebloom; Bull Mt. N. of Glendevey; Conglomerate, Lower Cloverly Fm., Cretaceous; Neg.
- A. lonchocarpus Torr., D319; tops; seed; Pagosa Springs; Shale, Mancos Fm., Cretaceous; Trace.
- A. pattersonii Gray, D157; tops; flower; Durango; Shale, Mancos Fm., Cretaceous; 1290 p.p.m., b.d.
- A. pattersonii Gray, D254; tops; flower; Durango; Shale, Mancos Fm., Cretaceous; 146 p.p.m., b.d.
- A. pattersonii Gray, D561; tops; seed; 5 mi. N. of Loma; Shale, Mancos Fm., Cretaceous; 181 p.p.m., a.d.

- A. pattersonii Gray, E51; tops; prebloom; Durango; Shale, Mancos Fm., Cretaceous; 382 p.p.m., b.d.
- A. pattersonii Gray, E487; tops; seed; 8 mi. S. of Kremmling; Shale, Pierre Fm.?, Cretaceous; 169 p.p.m., b.d.
- A. pattersonii Gray, T2; tops; seed; Ballback Farm near Fruita; Shale, Mancos Fm., Cretaceous; 5042 p.p.m., a.d.
- A. pattersonii Gray, T8; tops; seed; Meeker; Shale, Mancos Fm.?, Cretaceous; 288 p.p.m., a.d.
- A. pectinatus Dougl., Colo. 5; tops; seed; Apache Creek near Lascar; Shale, Niobrara Fm., Cretaceous; 1265 p.p.m., b.d.
- A. pectinatus Dougl., Colo. 14; tops; seed; Hugo; Shale, Pierre Fm., Cretaceous; 520 p.p.m., a.d.
- A. pectinatus Dougl., Colo. 17; tops; seed; near Bulger; Shale, Pierre Fm., Cretaceous; 502 p.p.m., b.d.
- A. pectinatus Dougl., Colo. 29; tops; seed; Hugo; Shale, Pierre Fm., Cretaceous; 660 p.p.m., b.d.
- A. pectinatus Dougl., Allred J; tops; seed; 2 mi. N. of Kremmling; Shale, Niobrara Fm., Cretaceous; 2114 p.p.m., a.d.
- A. racemosus Pursh, Colo. 3; tops; seed; Penrose; Shale, Niobrara Fm., Cretaceous; 530 p.p.m., b.d.
- A. racemosus Pursh, Colo. 12; tops; seed; Hugo; Shale, Pierre Fm., Cretaceous; 74 p.p.m., a.d.
- A. urceolatus Greene, D296; tops; seed; N.W. of Piedra; Shale, McDermott Grp., Cretaceous; 84 p.p.m., b.d.
- A. wingatanus Wats., E248; tops; seed; near Grand Junction; Shale, ——, Jurassic; Neg.
- Atriplex species, B423; tops; seed; 73 mi. W. of Craig; Shale, Morrison Fm., Upper Jurassic; 3 p.p.m., b.d.
- A. canescens (Pursh) Nutt., Allred 3; leaves & stems; seed; Fremont County; Shale, Morrison Fm., Upper Jurassic; trace.
- A. canescens (Pursh) Nutt., Allred 7; tops; seed; 10 mi.
  W. Pagosa Springs; Shale, Mancos Fm., Cretaceous;
  Nec.
- A. canescens (Pursh) Nutt., Colo. 7; tops; bud; Apache Creek near Lascar; Shale, Niobrara Fm., Cretaceous; 213 p.p.m., b.d.
- A. canescens (Pursh) Nutt., Colo. 8; tops; bud; Penrose; Shale, Niobrara Fm., Cretaceous; 6 p.p.m., b.d.
- A. canescens (Pursh) Nutt., Colo. 11; tops; seed; Hugo; Shale, Pierre Fm., Cretaceous; Neg.
- A. canescens (Pursh) Nutt., Colo. 20; tops; seed; N. of Wellington; Shale, Pierre Fm., Cretaceous; 6 p.p.m., b.d.
- A. canescens (Pursh) Nutt., Colo. 52; tops; seed; San Francisco Creek near Barela; Shale, Niobrara Fm., Cretaceous; 1 p.p.m., b.d.
- A. canescens (Pursh) Nutt., Colo. 53; tops; past seed;
   S. Walsenburg; Shale, Pierre Fm., Cretaccous; 3 p.p.m.,
   b.d.
- Berberis fendleri Gray, D236; leaves; seed; Durango; Shale, Mancos Fm., Cretaceous; Neg.
- Bouteloua gracilis (H.B.K.) Lag., Colo. 34; tops; seed; Jimmy Camp Creek near Colorado Springs; Shale, Pierre Fm., Cretaceous; 2 p.p.m., b.d.
- B. gracilis (H.B.K.) Lag., Colo. 37; tops; flower; Jimmy Camp Creek near Colorado Springs; Shale, Pierre Fm., Cretaceous; 23 p.p.m., b.d.
- B. gracilis (H.B.K.) Lag., Colo. 39; tops; seed; Jimmy Camp Creek near Colorado Springs; Shale, Pierre Fm., Cretaceous; 14 p.p.m., b.d.
- Chrysothamnus species, Colo. 48; tops; prebloom; ¼ mi. W. of Trinchera Creek near Trinchera; Shale, Benton Shale, Cretaceous; 1 p.p.m., b.d.
- Distichlis species, Colo. 23; tops; seed; N. of Wellington; Shale, Pierre Fm., Cretaceous; 15 p.p.m., b.d.

Eurotia lanata (Pursh.) Moq., Colo. 19; tops; seed; near Bulger; Shale, Pierre Fm., Cretaceous; 4 p.p.m., b.d.

(Irayia brandegei Gray, E508; tops; seeds; 20 mi. N. of Rifle; Shale, Wasatch Fm.?, Tertiary; 99 p.p.m., b.d.

Grindelia squarrosa (Pursh) Dunal, Allred A; tops; flower; Ridgway; Shale, Morrison Fm., Upper Jurassic; Neg.

G. squarrosa (Pursh) Dunal, Colo. 33; tops; flower; Jimmy Camp Creek near Colorado Springs; Shale, Pierre Fm., Cretaceous; 59 p.p.m., p.d.

Gaura coccinea Pursh, Allred C; tops; seed; Ridgway; Shale, Morrison Fm., Upper Jurassic; Neg.

Hedysarum species, E558; seeds & pods; seed; Colo. Nat. Monument near Grand Junction; Shale, —, Jurassic;

Hordeum jubatum L. & Sporobolus airoides Torr., Colo. 47; tops; bloom; ¼ mi. W. Trinchera Creek near Trinchera; Shale, Benton Shale, Cretaceous; 2 p.p.m., b.d.

Lactuca species, D350; tops; seed; N. of Pagosa Springs; Shale, ——, Cretaceous undivided; Neg.

Lupinus species, D212; flower & seed; seed; Durango; Shale, Mancos Fm., Cretaceous; Neg.

Lupinus species, D387; tops; bloom; Bull Mt. N. of Glendevey; Conglomerate, Lower Cloverly, Cretaceous; Neg.

Marrubium vulgare L., D239; tops; flower; Durango; Shale, Mancos, Cretaceous; Trace.

Medicago sativa L., D238; tops; flower; Durango; Shale, Mancos, Cretaceous; 5 p.p.m., a.d.

Melilotus alba Desv., Colo. 42; tops; flower; Jimmy Camp Creek near Colorado Springs; Shale, Pierre Fm., Cretaceous; 9 p.p.m., b.d.

Mentzelia nuda (Pursh) T. & G., Allred K; tops; flower; 3 mi. E. of Meeker; Shale, Mancos Fm.?, Cretaceous; 10 p.p.m., b.d.

Oonopsis engelmanii (Gray) Greene, Colo. 16; tops; bud; Hugo; Shale, Pierre Fm., Cretaceous; 123 p.p.m., a.d. O. engelmanii (Gray) Greene, Colo. 27; tops; flower;

Hugo; Shale, Pierre Fm., Cretaceous; 70 p.p.m., b.d.

O. engelmanii (Gray) Greene, D412; tops; seed; E. of

Limon; Shale, Pierre Fm., Cretaceous; 219 p.p.m., a.d.
 O. foliosa (Gray) Greene, Allred 6; tops; seed; Fremont County; Shale, Benton Shale, Cretaceous; Pos.

O. foliosa (Gray) Greene), Colo. 1a; tops; bud; Penrose; Shale, Niobrara Fm., Cretaceous; 164 p.p.m., b.d.

O. foliosa (Gray) Greene, Colo. 6; tops; bud; Apache Creek near Lascar; Shale, Niobrara Fm., Cretaceous; 3630 p.p.m., b.d.

O. foliosa (Gray) Greene, Colo. 36; tops; flower; Jimmy Camp Creek near Colorado Springs; Shale, Pierre Fm., Cretaceous; 140 p.p.m., b.d.

Oryzopsis hymenoides (R. & S.) Ricker, D255; tops; flower; Durango; Shale, Mancos Fm., Cretaceous; Neg.

O. hymenoides (R. & S.) Ricker, D257; tops; seed; Durango; Shale, Mancos Fm., Cretaceous; Trace.

Oxytenia acerosa Nutt., E506; tops; bloom; 20 mi. N.E. of Grand Junction; Shale and ss., Mesaverde Fm., Cretaceous; Neg.

Oxytropis saximontana A. Nels., D388; tops; bloom; Bull Mt. N. of Glendevey; Conglomerate, Lower Cloverly, Cretaceous; Neg.

saximontana A. Nels., Colo. 13; tops; seed; Hugo;
 Shale, Pierre Fm., Cretaceous; Neg.

Pachylophus hirsutus Rydb., D234; tops; flower; Durango; Shale, Mancos Fm., Cretaceous; Trace.

Pinus contorta murrayana Engelm., C650; leaves; —; Bull Mt. N. of Glendevy; Conglomerate, Lower Clovverly, Cretaceous; Neg.

P. flexilis James, C580; leaves; ——; Bull Mt. N. of Glendevey; Conglomerate, Lower Cloverly, Cretaceous; Neg.

Populus tremuloides Michx., C761; leaves; ——; Bull. Mt. N. of Glendevey; Conglomerate, Lower Cloverly, Cretaceous; Neg.

Purshia tridentata (Pursh) DC., E4; tops; winter; near Piedra; Shale, Mesaverde Grp.?, Cretaceous; 3 p.p.m., p.d.

Quercus species, D237; leaves; ——; Durango; Shale, Mancos Fm., Cretaceous; Neg.

Senecio species, E418; tops; bloom; Bull Mt. N. of Glendevey; Conglomerate, Lower Cloverly, Cretaceous; 4 p.p.m., b.d.

Sporobolus species, Colo. 32; tops; seed; 6 mi. E. of Colorado Springs; Shale, Lance Fm., Cretaceous; 2 p.p.m., p.d.

S. airoides Torr. & Hordeum jubatum L., Colo. 47; tops; bloom; ¼ mi. W. Trinchera Creek near Trinchera; Shale, Benton Shale, Cretaceous; 2 p.p.m., b.d.

S. airoides Torr.; tops; seed; Apache Creek near Lascar; Shale, Niobrara Fm., Cretaceous; 12 p.p.m., b.d.

Stanleya species, D558; tops; seed; 5 mi. N. of Loma; Shale, Mancos Fm., Cretaceous; 420 p.p.m., a.d.

S. species, T6; tops; past seed; Meeker; Shale, Dakota Grp., Cretaceous; 175 p.p.m., a.d.

S. species, T7; tops; past seed; W. of Grand Junction; Shale, Mancos Fm., Cretaceous; 823 p.p.m., a.d.

S. integrifolia James, T4; tops; seed; 7 mi. N.W. Fruita; Shale, Mancos Fm., Cretaceous; 130 p.p.m., a.d.

S. pinnata (Pursh) Britt., A38; tops; young; near Fort Collins; Shale, Morrison Fm., Upper Jurassic; Pos.

S. pinnata (Pursh) Britt., Colo. 2; tops; flower; Penrose; Shale, Niobrara Fm., Cretaceous; 15 p.p.m., b.d.

S. pinnata (Pursh) Britt., Colo. 9; tops; flower; Apache Creek near Lascar; Shale, Niobrara Fm., Cretaceous; 320 p.p.m., b.d.

S. pinnata (Pursh) Britt., Colo. 51; tops; bloom; 10 mi. W. Trinchera Creek near Trinchera; Shale, Niobrara Fm., Cretaceous; 37 p.p.m., b.d.

Stipa robusta Scribn., Allred 11; top; seed; 5 mi. W. of Pagosa Springs; Shale, Mancos Fm., Cretaceous; Neg.
S. robusta Scribn., Colo. 38; tops; flower; Jimmy Camp Creek near Colorado Springs; Shale, Pierre Fm., Cretaceous; 2 p.p.m., b.d.

Symphoricarpos oreophilus Gray, D235; leaves; flower; Durango; Shale, Mancos Fm., Cretaceous; Neg.

Taraxacum species, E420; tops; bloom; Bull Mt. N. of Glendevey; Conglomerate, Lower Cloverly, Cretaceous; 3 p.p.m., b.d.

Townsendia species, D240; tops; flower; Pagosa Springs; Shale, Mancos Fm., Cretaceous; Neg.

T. glabella Gray, D320; tops; seed; Pagosa Springs; Shale, Mancos Fm., Cretaceous; 3 p.p.m., a.d.

Triglochin maritima L., Colo. 24; tops; seed; South Park; Shale, Pierre Fm., Cretaceous; 12 p.p.m., b.d.

Triticum species, D567; grain; seed; near Durango; Shale, Mancos Fm., Cretaceous; 2 p.p.m., a.d.

Xylorrhiza glabriuscula Nutt., T5; tops; past seed; 7 mi. N.W. Fruita; Shale, Mancos Fm., Cretaceous; 4 p.p.m., a.d.

Zinnia species, Colo. 50; tops; bloom; 10 mi. W. Trinchera Creek near Trinchera; Shale, Niobrara Fm., Cretaceous; 9 p.p.m., b.d.

#### IDAHO

Two areas in Idaho have been examined critically by us for the distribution and kind of selenif-

erous vegetation. These areas were selected because of the occurrence on them of species of Stanleyas. In the Glenns Ferry section, seleniferous soil is confined exclusively to the Payette formation of Miocene age (Tertiary). The significant point to be kept in mind in the study of all seleniferous sedimentary formations of Tertiary age is that the source rock, of older age, from which the sediments were derived must have been seleniferous. Analyses of indicator plants from the Payette formation indicate that the selenium is distributed rather uniformly throughout the formation. The formations immediately below and above it are incapable of supporting seleniferous vegetation. The principal indicator plants on the Payette formation are Stanleya pinnata, S. bipinnata, and Astragalus toanus.

The second area near Winsper, Lemhi County, attracted our attention because of the abundance there of Stanleya viridiflora, Astragalus bisulcatus, and Aster adscendens. Reference to the data on these species shows a marked seleniferous condition particularly from the zone where the rocks are classified as massive limestones. Overlying the Paleozoic rocks are travertine deposits of Tertiary age. Indicator plants on the travertine deposits were much leaner in selenium than those growing on the limestones for the same stages of growth.

The influence of highly seleniferous plants upon associated vegetation is clearly demonstrated in this area. Attention is directed to the selenium values of many plant species not ordinarily capable of absorbing selenium unless made available to them. The principal contaminator plants in this area are Aster adscendens and Astragalus bisulcatus. The same plant species away from this immediate influence were usually non-seleniferous.

Soil samples from a cut bank below the limestone outcrop gave the 1st foot 1.13, 2nd foot 1.03, and the 3rd foot 0.79 parts per million selenium, respectively.

# Idaho Plant Samples

- Agropyron spicatum (Pursh) Scribn. and Smith, E462; tops; flower; 6 mi. S.E. of Twin Buttes; Igneous, ——, Quaternary?; Neg.
- Amsinckia intermedia Gray, E194; tops; flower; King Hill; Lake sediments, Payette Fm., Miocene; Neg.
- Artemisia spinescens D. C. Eaton, E209; tops; flower; 4 mi. S. of Castle Rock; Lava flow, —, Tertiary; Neg. Aster species, E188; tops; flower; Hagerman Draw; Lake sediments, Payette Fm., Miocene; Neg.
- A. species, E198; tops; flower; W. of Soda Springs; Igneous, Tertiary, Neg
- neous, —, Tertiary; Neg.

  Astragalus species, E215; tops; flower; Declo; Igneous,
- A. species (Probably new species, related to A. artemisiarum Jones and A. amplexus Payson), E211; tops; flower; 2 mi. W. of Mountain Home; Lake sediments, Payette Fm., Miocene; Neg.
- A. species (Same as above, probably new species), E212; tops; flower; King Hill; Lake sediments, Payette Fm., Miocene; Neg.
- Astragalus aculeatus A. Nels., E195; tops; flower; N.W. of Grand View; Lake sediments, Payette Fm., Miocene; Neg.

- A. amphioxys Gray, E191; tops; flower; Indian Cove; Lake sediments, Payette Fm., Miocene; Neg.
- A. curvicarpus (Sheld.) Macbr., E218; tops; flower; 2 mi. N.W. of Grand View; Lake sediments, Payette Fm., Miocene; Neg.
- A. diphysus Gray, T15; tops; past seeding; 67 mi. E. of Twin Falls; Alluvium, ——, Quaternary; Neg.
- A. lentiginosus Dougl. or A. heliophilus (Rydb.) Tides.,
   E187; tops; flower; 10 mi. E. of American Falls; Igneous, —, Tertiary; Neg.
- A. lonchocarpus Torr., E185; tops; flower; Bliss; Lake sediments, Payette Fm., Miocene; Neg.
- A. toanus Jones, E183; tops; flower; 3 mi. W. of Hammett; Lake sediments, Payette Fm., Miocene; 280 p.p.m., b.d.
- A. toanus Jones, E184; tops; flower; Bliss; Lake sediments, Payette Fm., Miocene; 622 p.p.m., b.d.
- A. utahensis T. & G., E204; tops; flower; 10 mi. W. of Wyoming line; Limestone, Thaynes Fm., Triassic; Neg.
- Atriplex species, E190; tops; seed; Hagerman Draw; Lake sediments, Payette Fm., Miocene; 4 p.p.m., b.d.
- Atriplex nuttallii S. Wats., E206; tops; flower; Orchard; Lava rock, —, Tertiary; Neg.
- Crepis acuminata Nutt., E217; tops; flower; 3 mi. W. of Hammett; Lake sediments, Payette Fm., Miocene; 2 p.p.m., a.d.
- Elymus condensatus Presl., E490; tops; flower; 10 mi. N.E. of Twin Buttes; Igneous, —, Quaternary?; Neg.
- Eurotia lanata (Pursh) Moq., E214; tops; flower; Bliss; Lake sediments, Payette Fm., Miocene; 5 p.p.m., a.d.
- Grayia spinosa (Hook.) Moq., E199; tops; flower; Bliss; Lake sediments, Payette, Miocene; 6 p.p.m., a.d.
- G. spinosa (Hook.) Moq., E220; tops; flower; 4 mi. S. of Castle Rock; Lava rock, —, Tertiary; Neg.
- Lithospermum species, E197; tops; flower; Bliss; Lake sediments, Payette Fm., Miocene; Neg.
- Mentzelia dispersa S. Wats., E189; tops; flower; Walters; Lake sediments, Payette Fm., Miocene; Neg.
- Oryzopsis species, E205; tops; flower; 2 mi. N.W. of Grand View; Lake sediments, Payette Fm., Miocene;
- Oryzopsis hymenoides (R. & S.) Ricker, E492; tops; flower; 10 mi. N.E. of Twin Buttes; Igneous, —, Quaternary?; Neg.
- Sitanion hystrix (Nutt.) J. G. Smith, E491; tops; flower; 10 mi. N.E. of Twin Buttes; Igneous, —, Quaternary?; Neg.
- Stanleya bipinnata Greene, E201; tops; flower; Bliss; Lake sediments, Payette Fm., Miocene; 364 p.p.m., b.d.
- S. bipinnata Greene, E207; tops; flower; King Hill; Lake sediments, Payette Fm., Miocene; 304 p.p.m., b.d.
- S. bipinnata Greene, T14; tops; seed; King Hill; Lake sediments, Payette Fm., Miocene, 352 p.p.m., a.d.
- S. pinnata (Pursh) Britt., E186; tops; flower; 11 mi. S.W. of Hammett; Lake sediments, Payette Fm., Miocene; 419 p.p.m., b.d.
- S. pinnata (Pursh) Britt., E196; tops; flower; N.W. of Grand View; Lake sediments, Payette Fm., Miocene; 53 p.p.m., b.d.
- S. pinnata (Pursh) Britt., E216; tops; flower; 3 mi. W. of Hammett; Lake sediments, Payette Fm., Miocene; 787 p.p.m., b.d.
- S. viridifiora Nutt., E301; tops; flower; Pocatello; Alluvium, —, Pleistocene; 2 p.p.m., b.d.
- S. viridifiora Nutt., E461; tops; flower; 6 mi. S.E. of Twin Buttes; Igneous, —, Quaternary?; 2 p.p.m., b.d.

- S. viridiflora Nutt., E489; tops; flower; 10 mi. N.E. of Twin Buttes; Igneous, ——, Quaternary?; 2 p.p.m., b.d.
- Tetradymia glabrata Gray, E193; tops; flower; near Bruneau; Lake sediments, Payette Fm., Miocene; Neg.
- T. glabrata Gray, E210; tops; flower; 3 mi. W. of Hammett; Lake sediments, Payette Fm., Miocene; Neg. T. spinosa H. & A., E213; tops; flower; Grand View;
- Lake sediments, Payette Fm., Miocene; 4 p.p.m., a.d.

  Thelypodium laciniatum var. milleftorum (A. Nels.) Pay-
- Townsendia florifer (Hook.) Gray, E208; tops; flower; King Hill; Lake sediments, Payette Fm., Miocene; Neg.

#### Near Winsper, Idaho

Agropyron species, E795; tops; seed; Meadows R. S. pasture; Massive limestone, —, Carboniferous; Neg. Agropyron smithii Rydb., E659; tops; seed; Warm Springs Creek; Massive limestone, —, Carboniferous; 15 p.p.m., p.d.

Artemisia tridentata Nutt., E663; leaves; seed; Warm Springs Creek; Massive limestone, ——, carboniferous;

4 p.p.m., a.d.

Aster species, E783; tops; seed; Warm Springs Creek; Massive limestone, —, Carboniferous; Neg.

- A. species, E785; tops; flower; Meadows R. S. pasture;
   Massive limestone, ——, Carboniferous; Neg.
- A. species, E790; tops; seed; Meadows R. S. pasture; Travertine, ——, Tertiary; Neg.
- A. species, E792; tops; seed; Meadows R. S. pasture; Travertine, —, Tertiary; Neg.
- A. species, E793; tops, seed; Meadows R. S. pasture; Travertine, —, Tertiary; 4 p.p.m., b.d.
- Aster adscendens Lindl., E664; tops; seed; Warm Springs Creek; Massive limestone, —, Carboniferous; 713 p.p.m., b.d.
- A. adscendens Lindl., E789; tops; past bloom; Meadows R. S. pasture; Travertine, —, Tertiary; 18 p.p.m., b.d.
- Astragalus bisulcatus (Hook.) Gray, E526; tops; flower; Meadows R. S. pasture; Massive limestone, —, Carboniferous; 2700 p.p.m., b.d.
- A. bisulcatus (Hook.) Gray, E657; tops; seed; Warm Springs Creek; Massive limestone, ——, Carboniferous; 190 p.p.m., b.d.
- A. bisulcatus (Hook.) Gray, E786; tops; past seed;
   Meadows R. S. pasture; Massive limestone, ——, Carboniferous; 122 p.p.m., b.d.
- A. bisulcatus (Hook.) Gray, E791; tops; seed; Meadows R. S. pasture; Massive limestone, —, Carboniferous; 755 p.p.m., b.d.
- A. bisulcatus (Hook.) Gray E797; tops; seed; Meadows R. S. pasture; Massive limestone, ——, Carboniferous; 410 p.p.m., b.d.
- Atriplex species, E784; leaves; flower; Meadows R. S. pasture; Massive limestone, —, Carboniferous; 2 p.p.m., b.d.
- Chenopodium species, E670; tops; seed; Warm Springs Creek; Massive limestone, —, Carboniferous; 15 p.p.m., a.d.
- Chenopodium album L., E662; tops; seed; Warm Springs Creek; Massive limestone, ——, Carboniferous; 52 p.p.m., b.d.
- Chrysothamnus nauseosus (Pallas) Britt., E660; tops; seed; Warm Springs Creek; Massive limestone, —, Carboniferous; 8 p.p.m., a.d.

Chrysothamnus viscidiflorus (Hook.) Nutt., E668; tops; seed; Warm Springs Creek; Massive limestone, —, Carboniferous; I p.p.m., a.d.

Cordylanthus ramosus Nutt., E667; tops; seed; Warm Springs Creek; Massive limestone, ——, Carboniferous; 2 p.p.m., a.d.

Elymus condensatus Presl., E788; tops; past seed; Meadows R. S. pasture; Travertine, —, Tertiary; Neg.

E. condensatus Presl., E527; tops; flower; Meadows R. S. pasture; Massive limestone, ——, Carboniferous; 13 p.p.m., b.d.

Eriogonum umbellatum Torr., E666; tops; seed; Warm Springs Creek; Massive limestone, ——, Carboniferous;

0.7 p.p.m., p.d.

Euphorbia esula L., E813; tops; flower; Meadows R. S. pasture; Travertine, —, Tertiary; Neg.

Eurotia lanata (Pursh) Moq., E658; tops; seed; Warm Springs Creek; Massive limestone, ——, Carboniferous; Neg.

Grass, E798; tops; seed; Meadows R. S. pasture; Travertine, ——, Tertiary; Neg.

Iva axillaris Pursh, E661; tops; seed; Warm Springs Creek; Massive limestone, —, Carboniferous; 30 p.p.m., b.d.

Iva axillaris Pursh, E782; tops; seed; Warm Springs Creek; Massive limestone, —, Carboniferous; 3 p.p.m., b.d.

Mentha canadensis L., E796; tops; flower; Meadows R. S. pasture; Massive limestone, —, Carboniferous; 5 p.p.m., b.d.

Poa secunda Presl., E529; tops; seed; Meadows R. S. pasture; Massive limestone, —, Carboniferous; 6 p.p.m., b.d.

Prunus melanocarpa (A. Nels.) Rydb., E787; leaves; past seed; Meadows R. S. pasture; Travertine, ——, Tertiary; Neg.

Stanleya viridiflora Nutt., E799; tops; dead plants; Meadows R. S. pasture; Travertine, —, Tertiary; 4 p.p.m., b.d.

S. riridiflora Nutt., E528; tops; seed; Meadows R. S. pasture; Massive limestone, ——, Carboniferous; 51 p.p.m., b.d.

S. viridiflora Nutt., E794; tops; dried out; Meadows R. S. pasture; Travertine, —, Tertiary; Neg.

Salsola pestifer A. Nels., E665; tops; seed; Warm Springs Creek; Massive limestone, ——, Carboniferous; 20 p.p.m., a.d.

Tetradymia canescens DC., E669; tops; seed; Warm Springs Creek; Massive limestone, ——, Carboniferous; 0.7 p.p.m., p.d.

Water Cress, E800; tops; flower; Warm Springs Creek; Travertine, —, Tertiary; 16 p.p.m., b.d.

# MONTANA

Exploratory surveys of indicator plants in Montana have been confined to two species, principally Astragalus bisulcatus and A. pectinatus. The main collections were made along highways from a point near Browning and continuing generally eastward to Shelby, Great Falls, Havre, Malta, Wolf Point, Circle, and Glendive. Twenty-seven samples of A. pectinatus and fifteen of A. bisulcatus were taken along this route. The stage of growth of the A. bisulcatus plants was largely past flower or early seed stage. Since A. pectinatus matures earlier than A. bisulcatus, our collections of it (July) included plants in the past seeding stages. Several samples

contained no fruiting heads. All of these Astragali were seleniferous.

The second area to be investigated was in the southeastern part of the state. Collections were made in the late fall; hence, the selenium results derived from these samples must be interpreted with this seasonal influence in mind.

Several geological formations capable of supporting seleniferous plants outcrop in Montana. In the areas investigated the principal formations belong to the Colorado group—namely, the Judith River, Claggett, Bearpaw, Lance, and Fort Union.

Colorado Group.—Upper Cretaceous in age. The generally accepted definition (21) of the Colorado Group includes only Benton and Niobrara and their equivalents. It was named for exposures at east base of the Rocky Mountain Front Range in Colorado.

Bearpaw, Claggett, and Judith River.—These formations are upper Cretaceous in age and in general are equivalent to the Steele, Mesaverde, and Lewis formations of south central Wyoming.

Lance.—The age of the Lance formation is in question. In eastern Montana it is believed to be upper Cretaceous.

Fort Union.—The Fort Union formation is of Eocene age (Paleocene or basal Eocene of some writers) and overlies the Lance.

The occurrence of seleniferous plants in these various geological formations in Montana is correlative with the seleniferous character of rocks deposited contemporaneously in Wyoming and other states. The selenium content of Astragalus pectinatus growing on the rocks and soils of the Fort Union formation establishes the continuity of its seleniferous character. This statement is substantiated by earlier work.

# Montana Plant Samples

- Aster species, D582; tops; past seed; 1 mi. N.E. of Fort Benton; Shale, Colorado Grp., Cretaceous; 10 p.p.m.,
- A. species, D583; tops; past seed; S. of Custer Battle-field; Alluvium, Lance, Cetraceous; 10 p.p.m., a.d.
- Aster commutatus T. & G., D586; heads; past seed; 12 mi. W. of Hardin; Shale, Colorado Grp., Cretaceous; Pos.
- Astragalus bisulcatus (Hook.) Gray, D844; leaves, past seed, Alzada; Shale, Pierre Fm., Cretaceous; 1550 p.p.m., a.d.
- A. bisulcatus (Hook.) Gray, ET42; tops; early seed; 17 mi. W. of Three Forks; —, —, ; 168 p.p.m., a.d.
- A. bisulcatus (Hook.) Gray, ET44; tops; flower; 9 mi. E. of Anaconda; —, —, —; 11 p.p.m., a.d.
- A. bisulcatus (Hook.) Gray, ET45; tops; flower; 8 mi. E. of Browning; Alluvium, —, Quaternary; 40 p.p.m., b.d.
- A. bisulcatus (Hook.) Gray, ET47; tops; early seed; Cutbank; Shale, Judith River?, Cretaceous; 53 p.p.m., a.d.
- A. bisulcatus (Hook.) Gray, ET50; tops; early seed; 8 mi. S. of Shelby; Shale, Colorado Grp., Cretaceous; 93 p.p.m., b.d.

- A. bisulcatus (Hook.) Gray, ET51; tops; early seed; 18
   mi. S. of Shelby; Shale, Colorado Grp., Cretaceous; 281
   p.p.m., a.d.
- A. bisulcatus (Hook.) Gray, ET53; tops; early seed; 5 mi. S. of Conrad; Shale, Colorado Grp., Cretaceous; 758 p.p.m., b.d.
- A. bivilcatus (Hook.) Gray, ET54; tops; early seed; 5 mi. S. of Conrad; Shale, Colorado Grp., Cretaceous; 142 p.p.m., b.d.
- A. bisulcatus (Hook.) Gray, ET55; tops; early seed; 7 mi. S. of Conrad; Shale, Colorado Grp., Cretaceous; 817 p.p.m., b.d.
- A. bisulcatus (Hook.) Gray, ET58; tops; flower; 8 mi. S. of Dutton; Shale, Colorado Grp., Cretaceous; 22 p.p.m., b.d.
- A. bisulcatus (Hook.) Gray, ET59; tops; seed; 8 mi.
   N.E. of Great Falls; Shale, Kotenai?, Cretaceous; 572
   p.p.m., b.d.
- A. bisulcatus (Hook.) Gray, ET61; tops; flower; 22 mi. N.E. of Great Falls; Shale, Kootenai?, Cretaceous; 2 p.p.m., b.d.
- A. bisulcatus (Hook.) Gray, ET62; tops; early seed; Carter; Shale, Colorado Grp., Cretaceous; 38 p.p.m., b.d.
- A. bisulcatus (Hook.) Gray, ET64; tops; seed; just N.E. of Fort Benton; Shale, Colorado Grp., Cretaceous; 82 p.p.m., b.d.
- A. bisulcatus (Hook.) Gray, ET67; tops; seed; 11 mi. N. of Havre; Shale, Judith River?, Cretaceous; 303 p.p.m., b.d.
- A. bisulcatus (Hook.) Gray, ET74; tops; early seed; 7 mi. W. of Hinsdale; Shale, Claggett, Cretaceous; 933 p.p.m., b.d.
- A. bisulcatus (Hook.) Gray, ET82; tops; seed; 9 mi. from Circle; Shale, ss., Fort Union, Tertiary; 52 p.p.m., b.d.
- A. canadensis L., ET70; tops; flower; 16 mi. E. of Harlem; Shale, Judith River, Cretaceous; 3 p.p.m., b.d.
- A. missouriensis Nutt., ET43; tops; seed; 17 mi. W. of Three Forks; —, —, Fore.
- A. pectinatus Dougl., D584; tops; past seed; 1 mi. N.E. of Fort Benton; Shale, Colorado Grp., Cretaceous; 57 p.p.m., a.d.
- A. pectinatus Dougl., D585; tops; past seed; 8 mi. N. of Culbertson; Shale, Fort Union, Tertiary; Pos.
- A. pectinatus Dougl., ET46; tops; early seed; 2 mi. W. of Cutbank; Shale, Judith River?, Cretaceous; 337 p.p.m., a.d.
- A. pectinatus Dougl., ET48; tops; seed; 3 mi. W. of Ethridge; Shale, Colorado Grp., Cretaceous; 217 p.p.m., a.d.
- A. pectinatus Dougl., ET49; tops; seed; 1 mi. S. of Shelby; Shale, Colorado Grp., Cretaceous; 166 p.p.m., a.d.
- A. pectinatus Dougl., ET52; tops; past seed; 4 mi. S. of Conrad; Shale, Colorado Grp., Cretaceous; 363 p.p.m., a.d.
- A. pectinatus Dougl. ET56; tops; seed; 7 mi. S. of Conrad; Shale, Colorado Grp., Cretaceous; 1815 p.p.m., b.d.
- A. pectinatus Dougl., ET57; tops; seed; 6 mi. S. of Dutton; Shale, Colorado Grp., Cretaceous; 410 p.p.m., b.d.
- A. pectinatus Dougl., ET60; tops; seed; 22 mi. N.E. of Great Falls; Shale, Kootenai?, Cretaceous; 89 p.p.m., b.d.
- A. pectinatus Dougl., ET63; tops; seed; 1 mi. E. of Fort Benton; Shale, Colorado Grp., Cretaceous; 685 p.p.m., hd
- A. pectinatus Dougl., ET65; tops; seed; 45 mi. S.W. of Havre; Shale, Claggett, Cretaceous; 761 p.p.m., a.d.

A. pectinatus Dougl., ET66; tops; seed; 20 mi. S.W. of Havre; Shale, Judith River, Cretaceous; 261 p.p.m., a.d.

 A. pectinatus Dougl., ET68; tops; seed; 5 mi. E. of Havre; Shale, Judith River, Cretaceous; 193 p.p.m., a.d.

A. pectinatus Dougl., ET69; tops; seed; 16 mi. E. of Havre; Shale, Judith River, Cretaceous; 1195 p.p.m., a.d.

A. pectinatus Dougl., ET71; tops; seed; 6 mi. E. of Dodson; Shale, Judith River, Cretaceous; 362 p.p.m., b.d.

A. pectinatus Dougl., ET72; tops; seed; 7 mi. E. of Dodson; Shale, Judith River, Cretaceous; 10 p.p.m., b.d.

A. pectinatus Dougl., ET73; tops; past seed; 10 mi. E. of Malta; Shale, Claggett, Cretaceous; 74 p.p.m., b.d.

A. pectinatus Dougl., ET75; tops; seed; 4 mi. E. of Frazer; Shale, Bearpow Fm., Cretaceous; 1703 p.p.m., b.d.

A. pectinatus Dougl., ET76; tops; seed; 2 mi. S.E. of Wolf Point; Shale, Lance?, Cretaceous; 777 p.p.m., b.d.

A. pectinatus Dougl., ET77; tops; past seed; 25 mi. S. of Wolf Point; Shale, Fort Union, Tertiary; 352 p.p.m., b.d.

A. pectinatus Dougl., ET78; tops; past seed; 26 mi. S. of Wolf Point; Shale, Fort Union, Tertiary; 46 p.p.m., b.d.

A. pectinatus Dougl., ET79; tops; seed; about 30 mi. S. of Wolf Point; Shale, Fort Union, Tertiary; 158 p.p.m., a.d.

A. pectinatus Dougl., ET80; tops; seed; 5 mi. from Circle; Shale, Fort Union, Tertiary; 543 p.p.m., b.d.

A. pectinatus Dougl., ET81; tops; seed; 9 mi. from Circle; Shale, Fort Union, Tertiary; 291 p.p.m., a.d.

A. pectinatus Dougl., ET83; tops; seed; 5 mi. S.E. of Lindsay; Shale, Fort Union, Tertiary; 628 p.p.m., a.d.

A. pectinatus Dougl., ET84; tops; past seed; 17 mi. E. of Glendive; Shale, Fort Union, Tertiary; 1385 p.p.m., a.d.

A. pectinatus Dougl., ET85; tops; past seed; E. of Glendive; Shale, Fort Union, Tertiary; 1220 p.p.m., a.d.

Barley and Oats, E377; grain; mature; near Billings; Shale, ——, Cretaceous; 2 p.p.m., a.d.

Stanleya pinnata (Pursh) Britt., D845; tops; past seed; Alvada; Shale, Pierre, Cretaceous; 112 p.p.m., a.d.

#### NEVADA

The authors became interested in the study of seleniferous indicator plants in Nevada because of the wide occurrence of the Stanleyas. In the late summer of 1937 collections of Stanleyas were made near the following towns: Wells, Battle Mountain, Fernley, and Winnemucca. Occurrences were noted at many other points along the highway particulary from Carson's Sink to Reno. The specimens collected were from old, badly weathered plants. Specific identifications were impossible in most instances for this reason. In 1938 (June) additional collections were made from other areas in Nevada. These collections were made near the following towns: Hawthorne, Crystal, Las Vegas, Ely, Caliente, Tonopah, Current, Mina, Hudson, Ludwig, and Lovelock.

The 1937 (D-samples) Stanleya collections were found by chemical analysis to be rather generally low in selenium. Because of the poor quality of specimens available to us, these low results should be interpreted with this point in mind. Surface soil samples were taken from some areas where the weathered Stanleyas grew. A few of these soil

analyses are given to illustrate the confusion that may develop in the interpretation of what constitutes a seleniferous area. Stanleya collections D-530, 531, 532, 533 and 534 gave only traces of selenium. Corresponding soil samples (surface 1 foot in depth) gave 1.22, 2.20, 1.20, none, and 1.25 parts per million selenium, respectively. It was also determined that in some Stanleya areas surface soil samples were negative for selenium, whereas the plants rooted in them (deeper soils) were seleniferous. These confusing results serve to illustrate the importance of soil sampling to the depth in which the plants are rooted.

It is realized that our brief exploratory investigations of the seleniferous status of indicator plants in Nevada are inadequate to evaluate even the areas referred to in this report. A few generalizations can be pointed out, however, as a result of our studies. One is that the Stanleyas as a whole are comparatively low in selenium. Whether this is due to a low soil selenium content or to environmental influences is not known to us. Indicator plants collected in the vicinity of the Toano Mountain Range in Elko County and the Shell Creek Range in White Pine County gave comparatively high selenium values. The seleniferous Astragali included: A. haydenianus, A. preussii, and A. toanus.

A complex series of rocks occur in Nevada. In a number of instances the geological formations are not known to us. Rocks of Cretaceous age or their equivalents are not represented in the areas dis-

cussed in this report.

In southeastern Nevada Astragalus preussii and Stanleya pinnata arcuata were collected from the detritus of massive limestones. These rocks are believed to be of Pennsylvanian age. In northern Nevada a sample of seleniferous Stanleya pinnata was taken from rocks assigned to the Humboldt formation of Tertiary age (Pliocene). This formation (11, 21) extends in patches from the west base of the Wasatch Mountains in Utah to the Humboldt River and Mountains in Nevada. Louderback (13) believes the Humboldt (Pliocene) and Lake Lohontan (Quaternary) lake beds are identical. Igneous rocks and sediments of Tertiary and of Quaternary age occupy many of the areas where Stanleya samples were taken. The source rocks from which the sediments were derived naturally would be older. however, the time (geological) of deposition determines the age of the formations.

It is believed that our correlation studies in Nevada support the general principle of the application of indicator plants in locating seleniferous soil areas. Further investigation should be made of the selenium content of the initial growth of the various

Stanleya species.

# Nevada Plant Samples

Astragalus species, E235; tops; flower; near Caliente;

A. species, E441; leaves; seed; E. of Wells; —, —, Carboniferous?; Neg.

Astragalus beckwithii T. & G., E318; tops; seed; 14 mi. S.E. of Ely; —, —, Carboniferous?; Neg.

A. beckwithii T. & G., E323; tops; seed; 25.5 mi. S.W. of Wendover; Shale, —, Carboniferous?; 5 p.p.m., a.d. Astragalus canonis Jones, E305; tops; seed; 9 mi. W. of Hawthorne; —, Jurassic?; 2 p.p.m., b.d.

Astragalus canadensis L., E309; tops; seed; Big Indian Canyon; —, —, Jurassic?; Neg.

A. canadensis L., E315; tops; seed; Gardnerville; Alluvium, —, Quaternary; Neg.

Astragalus diphysus Gray, D559; tops; old plants; 15 mi. E. of Reno; Igneous, —, Tertiary; 4 p.p.m., a.d. Astragalus haydenianus Gray, E304; tops; seed; 14 mi.

S.E. of Ely; Alluvium, —, Quaternary; 631 p.p.m.,

Astragalus lentiginosus Dougl., E297; tops; early seed; Caliente; —, —, ; Neg.

A. lentiginosus Dougl., E298; tops; early seed; Caliente; \_\_\_\_\_, \_\_\_\_; 9 p.p.m., b.d.

A. lentiginosus Dougl., E299; tops; early seed; Caliente;

A. lentiginosus Dougl., E306; tops; seed; 2 mi. W. of Mina; Alluvium, —, Quaternary; Neg.

A. lentiginosus Dougl., E314; tops; seed; 2 mi. S.W. of Wendover; Alluvium, —, Quaternary; Neg.

A. lentiginosus Dougl., E317; tops; seed; 25 ml. S.W. of Wendover; Shale, ——, Carboniferous?; 1 p.p.m., b.d.

Astragalus preussii Gray, E110; tops; seed; 8 mi. from Las Vegas; Massive limestone, —, Carboniferous; 27 p.p.m., a.d.

Astragalus toanus Jones, E310; tops; seed; 25.5 mi. S.W. of Wendover; Shale, ——, Carboniferous; 265 p.p.m., b.d.

Astragalus utahensis T. & G., E327; tops; seed; 14 mi. S.E. of Ely; Shale, —, Carboniferous?; 2 p.p.m., a.d. Encelia frutescens Gray, E112; tops; flower; between

Boulder City and Boulder Dam; Igneous, —, Tertiary; Neg.

Stanleya species, D528; tops; old plants; 2.6 mi. W. of Wells; Shale (sandy), Humboldt Fm., Pliocene; 41 p.p.m., a.d.

Stanleya?, D531; tops; old plants; 30 mi. W. of Battle Mountain; Igneous, —, Tertiary; Neg.

S. species, D532; tops; old plants; 31.5 mi. W. of Battle Mountain; Igneous, ——, Tertiary; Trace.

S. species, D533; tops; old plants; 39 mi. E. of Fernley; Alluvium, —, Quaternary; Trace.

S. species, D534; tops; old plants; 25.6 mi. E. of Fernley; Igneous, —, Tertiary; Trace.

S. species, D556; tops; old plants; 26 mi. E. of Winnemucca; Alluvium, —, Quaternary; Neg.

S. species, D562; tops; old plants; 8 mi. E. of Wells;

S. species, E313; tops; seed; 25.5 mi. S.W. of Wendover; Shale, —, Carboniferous?; 116 p.p.m., b.d.

S. species, E320; tops; seed; 1 mi. E. of Currant; Alluvium, —, Quaternary; 2 p.p.m., b.d.

S. species, E322; tops; seed; 3 mi. N.W. of Tonopah; Igneous, —, Tertiary; 4 p.p.m., a.d.

S. species, E325; tops; seed; 26 mi. W. by S. of Currant; Igneous, —, Tertiary; 3 p.p.m., a.d.

S. species, E326; tops; seed; 3½ mi. S.W. of Wendover; Alluvium, —, Quaternary; 4 p.p.m., a.d.

S. species, E444; leaves; seed; 4 mi. W. of Hawthorne; Alluvium, —, Quaternary; 3 p.p.m., a.d.

Stanleya pinnata (Pursh) Britt., E120; tops; flower; near Crystal; Massive limestone, —, Carboniferous; 30 p.p.m., b.d.

S. pinnata (Pursh) Britt., E255; tops; flower; near Caliente; —, —, ; 66 p.p.m., b.d.

S. pinnata (Pursh) Britt., E296; leaves to seeds; late flower; near Caliente; —, —, ; 4 p.p.m., b.d.

S. pinnata (Pursh) Britt., E312; tops; seed; 8 mi. W. of Hawthorne; Alluvium, —, Quaternary; 3 p.p.m., b.d.

S. pinnata (Pursh) Britt., E316; tops; seed; 28 mi. E. of Tonopah; Alluvium, —, Quaternary; 3 p.p.m., b.d.

S. pinnata (Pursh) Britt., E321; tops; seed; 7.5 mi. N. of Mina; Alluvium, —, Quaternary; 75 p.p.m., a.d.

S. pinnata (Pursh) Britt., E409; leaves; seed; near Hudson; Alluvium, —, Quaternary; 2 p.p.m., a.d.

S. pinnata (Pursh) Britt., E410; leaves; seed; near Ludwig; Alluvium, —, Quaternary; 66 p.p.m., b.d.

S. pinnata (Pursh) Britt., E440; leaves; seed; 35 mi. W. of Lovelock; Igneous, —, Tertiary; 11 p.p.m., a.d.

S. pinnata (Pursh) Britt., E443; leaves; seed; Rye Patch Dam; Alluvium, Lake Lohontan sediment, Quaternary; 5 p.p.m., a.d.

Stanleya pinnata arcuata (Pursh) Britt., E111; tops; flower; 8-9 mi. from Las Vegas; Massive limestone, —, Carboniferous; 9 p.p.m., a.d.

Stanleya viridiflora Nutt., D529; tops; old plants; 33 mi. W. of Winnemucca; Alluvium, —, Quaternary; 28 p.p.m., p.d.

S. viridiffora? Nutt., D530; tops; old plants; 5 mi. W. of Wendover; Alluvium, —, Quaternary; Trace.

S. viridiflora? Nutt., D555; tops; old plants; 23 mi. S.W. of Lovelock; Alluvium, —, Quaternary; Pos.

S. viridiflora Nutt., E307; tops; seed; 2 mi. W. of Mina; Alluvium, —, Quaternary; 2 p.p.m., a.d.

S. viridiftora Nutt., E311; tops; seed; 9 mi. W. of Hawthorne; —, —, Jurassic?; 1 p.p.m., b.d.

S. viridiflora Nutt., E328; tops; seed; 28 mi. E. of Tonopah; —, —, —; 4 p.p.m., b.d.

Tetradymia glabrata Gray, E324; tops; seed; 2 mi. S.W. of Wendover; Alluvium, —, Quaternary; Neg.

# NEW MEXICO

Plant collections in New Mexico have been made from numerous rock types. Many samples were taken regardless of soil relationships, particularly species of Astragalus. A detailed study was made of the seleniferous plants indigenous to the area just north of Springer, New Mexico. About 30,000 acres of range and irrigated land was included in the survey. The data have been tabulated separately from the other New Mexico areas.

Considerable attention was given to vegetation occurring on soils derived from rocks mapped as Cretaceous and Tertiary in age. A type exposure occurs in the Magdalena section west of Socorro. Marsh and Clawson (14) had indicated from reports reaching them that Astragalus praelongus occurred in the Magdalena region. It was believed to be poisonous to sheep. Since A. praelongus is one of the dominant selenium-absorbing species, it was sought for in this area. Several excursions were made into the Magdalena section during 1938. No Astragali corresponding to praelongus could be found. Marsh and Clawson's (14) statement that Astragalus praelongus and A. sabulosus are synony-

mous is in error.2 Each is a valid type species—A. praelongus Sheld. and A. sabulosus Jones. The latter (10) has a very restricted distribution, being confined largely to its type locality in eastern Utah. Additional search for seleniferous indicator plants on the Tertiary volcanic rocks was continued from Socorro to Las Cruces. None were found. .

In so far as our investigations have gone, it is evident that the geological formations capable of supporting seleniferous vegetation in New Mexico are referable to rock types of Triassic, Cretaceous, and Tertiary age. The principal seleniferous areas

occur in northern New Mexico.

Our field surveys were not planned to cover completely the various geological formations that might support seleniferous vegetation. Many of the formations were not identified in the field. In most instances of this kind the age and rock type have been given.

The geological formations of New Mexico found to be associated with the selenium problem include Mancos shale, Cretaceous undivided, Mesaverde,

and rocks of Tertiary age.

Mancos shale.—The marine mancos shale has vielded faunas (18) of, in ascending order, Green-

horn, Carlile, Niobrara and Pierre age.

Mesaverde Group (also formation).—The typical Mesaverde formation (17) is wholly of Pierre age. It is yet to be determined if this formation is genetically traceable into all the states in which the name has been applied. The Mesaverde formation of southeastern Wyoming is divisible into the marine (lower) and non-marine (upper) members. The marine portion is capable of supporting seleniferous vegetation. The non-marine is not. Whether or not this generalization can be carried into other sections of Wyoming or into other states is not

Pierre-Benton.—These rocks are Cretaceous in age. The name includes the Pierre formation and the Benton shale. Rock types carrying this generalized nomenclature have not been divided into lithological divisions. Seleniferous vegetation collected from areas bearing the name Pierre-Benton cannot be definitely associated with an individual formation such as Thermopolis, Mowry, Frontier, or Pierre. Hence, these rocks are referred to the Cretaceous undivided.

Pierre-Niobrara-Benton .- An assemblage of rocks in which representative formations have not been mapped. It is impossible to state, in areas so classified, whether seleniferous plants were growing on soils of a Niobrara character or some other

type rock falling within the assemblage.

Indicator plants collected from type localities characterized by rocks of Tertiary age in the Aztec-Regina section of New Mexico are seleniferous. The occurrence of seleniferous vegetation on rocks of Tertiary age have not been determined in other areas. In east central New Mexico rocks of Permian

<sup>2</sup> The types have been examined by Dr. Aven Nelson, University of Wyoming. He is satisfied that they are distinct species.

and Triassic age were explored for seleniferous plants early in the spring of 1937. The vegetation was not far enough advanced for adequate sampling. However, the few specimens secured were not found to be selenium bearing. Additional information is needed before conclusions can be drawn regarding the seleniferous character of the rocks of late Permian and lower Triassic age in this area.

A careful investigation of the seleniferous character of the native plants indigenous to the Springer area was undertaken to determine (1) the principal indicator plants, (2) the areas where native grasses were seleniferous, (3) the influence, if any, that native seleniferous plants were exerting on associ-

ated forages and farm crops.

The dominant seleniferous plants of this area are the Astragali. The native grasses are toxic only where these contaminator plants have caused the soil to be enriched with available selenium. Dense stands of Astragalus pattersonii, A. bisulcatus, and A. praelongus occur in the grassy vegas (moist swales). Grass sample, D-365, was taken from one of these vegas. Grass sample D-263 was taken from the edge of irrigated meadow but was growing near seleniferous Astragalus bisulcatus. Grass (same species) sample D-274 was taken from the same meadow but away from the Astragalus influence.

Since the time of this survey the bulk of the seleniferous Astragali in the area inspected have been grubbed out and burned in order to remove the menace that had caused the grasses and other valuable forages to be toxic to livestock. A decided improvement in the livestock in this section was

noted in 1938.

# New Mexico Plant Samples

Astragalus species, D104; tops; prebloom; near Fort Sumner; Shale, Dockum Grp., Triassic; Neg.

A. species, E70; tops; prebloom; S. of Santa Fe; Arkose, Shale, Santa Fe Fm., Tertiary; Neg.

A. species, E71; tops; prebloom; 7 mi. from Santa Fe; Arkose, Shale, Santa Fe Fm., Tertiary; Neg.

A. species, E76; tops; prebloom; S. of Magdalena; Igneous, ---, Cret. & Tert.; Neg.

Astragalus amphioxys Gray, E229; tops; seed; Ruidoso; Shale, Chupadera Fm., Permian; Neg.

A. confertiflorus Gray, E374; tops; prebloom; 45 mi. S. of Aztec; Shale, ---, Eocene; 143 p.p.m., b.d.

A. diphysus Gray, E73; tops; prebloom; Socorro; Igneous, ---, Tertiary; Neg.

A. diphysus Gray, E75; tops; prebloom; 5 mi. W. of Magdalena; Igneous, ---, Cret. & Tert.; Neg.

A. diphysus Gray, E231; tops; flower; W. of Magdalena; Alluvium, ----, Quaternary; 1 p.p.m., a.d.

A. fendleri Gray, E773; tops; seeds; Beaverhead; Al-

luvium, ----, Quaternary; Neg.

A. haydenianus Gray, E344; tops; seed; 85 mi. N. of Santa Fe; Shale, Pierre-Benton, Cret. undiv.; 53 p.p.m.,

A. haydenianus Gray, E375; tops; past seed; Cuba; Shale, -, Tertiary; 13 p.p.m., b.d.

A. humistratus Gray, E228; tops; seed; Ruidoso; Shale, Chupadera Fm., Permian; Neg.

A. humistratus Gray, E618; tops; seed; Tilden S. of Cuba; Shale, Mesaverde?, Cretaceous; Neg.

A. humistratus Gray, E653; tops; seed; Beaverhead; Alluvium, —, Quaternary; Neg.

A. lentiginosus Dougl., E77; tops; prebloom; 25 mi. N. of Hot Springs; Igneous, —, Cret. & Tert.; Neg.

A. lentiginosus Dougl., E85; tops; seed; near Virden; Alluvium, ——, Quaternary; Neg.

A. lentiginosus Dougl., E101; tops; seed; Gage; Alluvium, —, Quaternary; Neg.

A. lonchocarpus Torr., E345; tops; seed; 81 mi. N. of Santa Fe; Shale, Pierre-Benton, Cret. undiv.; 2 p.p.m., b.d.

A. lonchocarpus Torr., E365; tops; seed; Santa Fe; Shale, —, Tertiary; Pos.

A. mollissimus Torr., D102; tops; flower; 22 mi. N. of Fort Sumner; Shale, Dockum Grp., Triassic; Neg.

A. nuttallianus DC., E243; tops; seed; 6 mi. S. of Shiprock; Shale, Mancos Fm., Cretaceous; Neg.

 A. oocalycis Jones, E373; tops; prebloom; 50 mi. S. of Aztec; Shale, —, Tertiary; 413 p.p.m., a.d.

A. oocalycis Jones, E617; tops; seed; 1.2 mi. N. of Re-

gina; Shale, —, Eocene; 42 p.p.m., b.d.

A. pattersonii Gray, E182; tops; prebloom; 18 mi. N. of
Albuquerque; Shale, —, Tertiary; 11 p.p.m., b.d.

A. pattersonii Gray, E244; tops; seed; N. of Gallup; Shale, ss., coal, Mesaverde?, Cretaceous; 676 p.p.m., b.d.

A. pattersonii Gray, E442; tops; seed; 3 mi. N. of San Jon; Shale, Dockum Grp., Triassic; 15 p.p.m., b.d.

A. praelongus Sheld., C142; tops; flower; Colfax; Shale, Pierre-Niob.-Bent., Cret. undiv.; 1352 p.p.m., a.d.

A. praelongus Sheld., C143; tops; prebloom; 4 mi. from Raton; Shale, Pierre-Niob.-Bent., Cret. undiv.; 4474 p.p.m., a.d.

A. praelongus Sheld., E72; tops; prebloom; 25 mi. from Albuquerque; Alluvium, —, Quaternary; 262 p.p.m., a.d.

A. praelongus Sheld., E376; tops; flower; 15 mi. S. of Crown Point; Shale, ss., Mancos Fm.?, Cretaceous; 388 p.p.m., b.d.

A. preussii arctus Sheld., E363; tops; seed; 20 mi. S. of Santa Fe; Igneous, —, Tertiary; 774 p.p.m., b.d.

A. tenellus Pursh, E364; tops; seed; Santa Fe; Igneous, —, Tertiary; Neg.

A. thurberi Gray, E151; tops; seed; 10 mi. E. of Hachita; Alluvium, —, Quaternary; Neg.

A. wootoni Sheld., E78; tops; seed; 5 mi. S. of Hot Springs; Alluvium, —, Quaternary; Neg.

A. wootoni Sheld., E152; tops; seed; 10 mi. E. of Hachita; Alluvium, —, Quaternary; Neg.

A. wootoni Sheld., E232; tops; seed; 5 mi. E. of Magdalena; Alluvium, —, Quaternary; Neg.

Caulanthus lasiophyllus Payson, E84; tops; seed; Mesilla Park; Alluvium, —, Quaternary; Neg.

Euphorbia marginata Pursh, D780; tops; flower; Roy; Sandstone, Dakota Grp., Cretaceous; Neg.

Grass, D97; tops; prebloom; 26 mi. N.W. of Fort Sumner; Shale, limestone-ss., Dockum Grp., Triassic; Neg. Larrea tridentata (DC.) Vail, E80; tops; flower; 20 mi. N. of Las Cruces; Volcanic residues, ——, Cret. & Tert.; Neg.

Mentzelia species, E74; roots; prebloom; Magdalena; Alluvium, —, Quaternary; Neg.

Mentzelia multiflora (Nutt.) Gray, E79; tops; flower; N. of Las Cruces; Igneous, —, Cret. & Tert.; Neg.

Oxytropis saximontana A. Nels., E230; tops; seed; E. of Mescallero; Shale, Chupadera Fm., Permian; Neg. Senecio species, D779; tops; flower; Roy; Sandstone, Da-

kota Grp., Cretaceous; Neg.

Sophora sericea Nutt., E227; tops; seed; W. of Carrizozo; Shale, Chupadera Fm., Permian; Neg.

Stanleya pinnata (Pursh) Britt., E250; tops; seed; S. of Shiprock; Shale, Mancos Fm., Cretaceous; 35 p.p.m., b.d.

Stanleya pinnata (Pursh) Britt., D366; tops; flower; Philmont Ranch; Shale, Pierre-Benton, Cret. undiv.; 1110 p.p.m., b.d.

#### Springer Area, New Mexico

The following samples were all collected from near Springer, New Mexico, where they grew on Pierre-Benton shale of Cretaceous age. The above ground portions of the plants were analyzed.

Aster species, D356; flower; 15 p.p.m., b.d. Astragalus bisulcatus (Hook.) Gray, D245; prebloom; 1321 p.p.m., b.d.

A. haydenianus Gray, D379; seed; 185 p.p.m., a.d.
A. praelongus Sheld., D246; flower; 2600 p.p.m., b.d.

A. praelongus Sheld., D362; seed; 1030 p.p.m., a.d. A. racemosus Pursh, D360; flower; 113 p.p.m., b.d.

A. serpens Jones, D364; seed; Neg. Bahia oppositifolia Nutt., D355; flower; Neg. Bouteloua species, D365; seed; 46 p.p.m., a.d. Euphorbia species, D414; flower; Neg.

Grass, D263; flower; 11 p.p.m., a.d. Grass, D274; flower; Trace.

Grindelia species, D358; flower; 18 p.p.m., b.d. Helianthus species, D598; prebloom; Neg. H. species, D599; prebloom; 6 p.p.m., a.d. Hordeum species, D357; flower; Neg.

Iva species, D363; seed; Neg.

Lycium species, D359; flower; 3 p.p.m., b.d.

Medicayo sativa L., eight samples; flower; Neg.

Native Hay, nine samples No no.; seed; Neg.

Native Hay, one sample No no.; seed; 3 p.p.m., a.d.

Oat Hay, D724; prebloom; Neg.

Sophora sericea Nutt., D247; flower; Trace.

# NORTH DAKOTA

A composite sample of Astragalus pectinatus, past seed stage, collected near Beach was seleniferous. Upon analysis it was found to contain 924 parts per million selenium on an air dry basis. The geological formation dominant in the Beach area is the Fort Union (Tertiary). The seleniferous character of A. pectinatus in this section of North Dakota is similar to that obtained for the A. pectinatus samples in eastern Montana on the Fort Union formation and for the same stage of growth.

# OREGON

Dried specimens of Stanleya viridiflora from the Steen Mountain section of southeastern Oregon were seleniferous. In view of the fact that S. viridiflora is generally low in selenium, even on highly seleniferous soils, it is concluded that a more careful study of the indigenous vegetation of this region would be necessary before definite conclusions could be arrived at. The character of the rocks in which the S. viridiflora grew is not known to us.

A few miscellaneous Astragalus specimens from various parts of Oregon were found not to be seleniferous. These included A. sclerocarpus, A. reventors, A. succumbens, and A. howelli. Not any of these Astragali, however, belong to the selenium-absorbing groups.

#### SOUTH DAKOTA

The most dominant and widely distributed native, seleniferous plants of South Dakota are the Asters. The two principal species so far reported on (1, 3, 5) are Aster commutatis T. & G. and A. ericoides L. (Aster multiflorus). There may be additional species still to be determined. The Asters are late in maturing; consequently it is difficult to identify collections previous to the flowering stage.

The seleniferous Astragalus racemosus, A. bisulcatus, and A. pectinatus are rather generally reliable indicators of selenium-bearing soils. A. racemosus has a wider distribution than has A. pectinatus or A. bisulcatus in the areas inspected by the authors. The Stanleya species are excellent indicators of a seleniferous soil, although they are scattered in their distribution. Gutierrezia sarothrae (Pursh) Britt. and Rusby (G. euthamiae T. & G.) has been reported by Moxon (16) and Byers (6) to be rather generally seleniferous. It has a wide latitude of growth and like the Asters may occur on soils free of selenium. Also on some highly seleniferous raw shales, the authors have found this species incapable of absorbing selenium in significant quantities.

Oonopsis argillacea and Xylorrhiza glabriuscula of southwestern South Dakota are confined exclu-

sively to seleniferous soils.

It is not our purpose to review all the plants of this state or any other as to their seleniferous status. We merely wish to point out that certain native plants stand out in their selenium-absorbing properties and that this property is associated with definite geological formations.

The seleniferous areas in South Dakota are associated with formations of Cretaceous and Tertiary age. The Pierre shale (Cretaceous) is the most important exposure in so far as seleniferous soils and plants are concerned. The Fox Hills sandstone and the Pierre formation are stratigraphically equivalent in age to the Steele, Mesaverde, and Lewis formations of central Wyoming.

# South Dakota Plant Samples

Aster species, D196; tops; young; 14 mi. N. of Bonesteel; Shale, Pierre Fm., Cretaceous; 59 p.p.m., a.d.

Aster species, D201; tops; young; 14 mi. N. of Bonesteel; Shale, Pierre Fm., Cretaceous; Pos.

Aster species, D202; tops; young; 14 mi. N. of Bonesteel; Shale, Pierre Fm., Cretaceous; 21 p.p.m., a.d.

Aster species, D203; tops; young; 14 mi. N. of Bonesteel; Shale, Pierre Fm., Cretaceous; 83 p.p.m., a.d.

- -Aster commutatus T. & G., D167; tops; young; 20 mi. S.E. of Pierre; Shale, Pierre Fm., Cretaceous; 335 p.p.m., a.d.
- A. commutatus T. & G., D182; tops young; 2 mi. out of Midland; Shale, Pierre Fm., Cretaceous; 282 p.p.m., a.d.
- A. commutatus T. & D., D872; tops; prebloom; Central part of State; Shale, Pierre Fm., Cretaceous; 419 p.p.m., a.d.
- A. ericoides L.,\* D184; tops; young; 14 mi. S. of Pierre (Dean farm); Shale, Pierre Fm., Cretaceous; 80 p.p.m., a.d.

- A. ericoides L.,\* D193; tops; flower; 6 mi. W. of Hot Springs; Shales, Spearfish Fm., Jurassic?; 2 p.p.m., a.d.
   A. ericoides L.,\* D194; tops; flower; 6 mi. E. of Hot Springs; Shale, Spearfish Fm., Jurassic?; Trace.
- A. ericoides L.,\* D207; tops; young; 20 mi. S.E. of Pierre (Reed farm); Shale, Pierre Fm., Cretaceous; 440 p.p.m., a.d.
- Astragalus species, D172; tops; flower; 20 mi. S.E. of Pierre (Reed farm); Shale, Pierre Fm., Cretaceous; 1610 p.p.m., a.d.
- A. species, D181; tops; young; 2 mi. out of Midland; Shale, Pierre Fm., Cretaceous; Neg.
- A. species, ET20; tops; past flower; 18 mi. S. of Pierre; Shale, Pierre Fm., Cretaceous; 3 p.p.m., a.d.
- A. flexuosus Dougl., ET11; tops; past flower; 15 mi. S. of Pierre; Shale, Pierre Fm., Cretaceous; 6 p.p.m., a.d.
- A. nitidus Dougl., ET7; tops; flowers; 7 mi. N. of Vivian; Shale, Pierre Fm., Cretaceous; 9 p.p.m., a.d.
- A. nitidus Dougl., ET10; tops; flower; 15 mi. S. of Pierre; Shale, Pierre Fm., Cretaceous; 2 p.p.m., a.d.
- Astragalus pectinatus Dougl., D208; tops; flower; E. of Pine Ridge; —, —, ; 3116 p.p.m., a.d.
- A. racemosus Pursh, D163; tops; young; 20 mi. S.E. of Pierre; Shale, Pierre Fm., Cretaceous; 2210 p.p.m., a.d.
- A. racemosus Pursh, D195; tops; flower; 12 mi. from Bonesteel toward Mulehead Ranch; Shale, Pierre Fm., Cretaceous; Pos.
- A. racemosus Pursh, ET1; tops; past flower; 6 mi. W. of Yankton; Shale, Niobrara Fm., Cretaceous; 40 p.p.m., a.d.
- A. racemosus Pursh, ET5; tops; flower; 4 mi. N. of Vivian; Shale, Pierre Fm., Cretaceous; 11 p.p.m., a.d.
- A. racemosus Pursh, ET6; tops; past flower; 7 mi. N. of Vivian; Shale, Pierre Fm., Cretaceous; 13 p.p.m., a.d.
- A. racemosus Pursh, ET8; tops; past flower; 5 mi. N. of Pierre; Shale, Pierre Fm., Cretaceous; 75 p.p.m., a.d.
- A. racemosus Pursh, ET9; tops; flower; 15 mi. S. of Pierre; Shale, Pierre Fm., Cretaceous; 445 p.p.m., a.d.
- A. racemosus Pursh, ET12; tops; flower; 15 mi. S. of Pierre; Shale, Pierre Fm., Cretaceous; 3920 p.p.m., a.d.
- A. racemosus Pursh, ET13; tops; past flower; 13 mi. S. of Pierre; Shale, Pierre Fm., Cretaceous; 188 p.p.m., a.d.
- A. racemosus Pursh, ET14; tops; past flower; 15 mi. S. of Pierre; Shale, Pierre Fm., Cretaceous; 34 p.p.m., a.d.
- A. racemosus Pursh, ET15; tops; past flower; 16 mi. S. of Pierre; Shale, Pierre Fm., Cretaceous; 40 p.p.m., a.d.
- A. racemosus Pursh, ET16; tops; past flower; 17 mi. S. of Pierre; Shale, Pierre Fm., Cretaceous; 21 p.p.m., a.d.
- A. racemosus Pursh, ET18; tops; past flower; 17 mi. S. of Pierre; Shale, Pierre Fm., Cretaceous; 147 p.p.m.,
- A. racemosus Pursh, ET19; tops; past flower; 18 mi. S. of Pierre; Shale, Pierre Fm., Cretaceous; 410 p.p.m., a.d.
- A. racemosus Pursh, ET21; tops; past flower; 14 mi. S. of Pierre; Shale, Pierre Fm., Cretaceous; 2743 p.p.m., a.d.
- A. racemosus Pursh, ET22; tops; past flower; 14 mi. S. of Pierre; Shale, Pierre Fm., Cretaceous; 280 p.p.m., a.d.
- A. racemosus Pursh, ET23; tops; past flower; 1 mi. N. of Draper; Shale, Pierre Fm., Cretaceous; 81 p.p.m., a.d.
- A. racemosus Pursh, ET24; tops; past flower; 6 mi. W. of Kadoka; Shale, Chadron Fm.?, Tertiary; 91 p.p.m., a.d.
  - \* Aster multiflorus Ait.

- A. racemosus Pursh, ET25; tops; past flower; 7 mi. W. of Kadoka; Shale, Chadron Fm.?, Tertiary; 77 p.p.m., a.d.
- A. racemosus Pursh, ET26; tops; flower; 4 mi. E. of Cottonwood; Shale, Pierre?, Cretaceous; 1330 p.p.m., a.d.
- A. racemosus Pursh, ET27; tops; past flower; 3 mi. E. of Wall; Shale, Chadron Fm.?, Tertiary; 8 p.p.m., a.d.
- Avena species (oats) 1937, D826; grain; seed; Rumford; Shale, Pierre Fm., Cretaceous; 14 p.p.m., a.d.
- Avena species (oats) 1938, E69; grain; seed; Rumford; Shale, Pierre Fm., Cretaceous; 4 p.p.m., a.d.
- Glycyrrhiza lepidota Pursh, ET17; tops; flower; 17 mi. S. of Pierre; Shale, Pierre Fm., Cretaceous; 2 p.p.m., ad
- Grass, D164; tops; young; S. of Pierre 20 mi.; Shale, Pierre Fm., Cretaceous; 18 p.p.m., a.d.
- Grass, D166; tops; young; 20 mi. S.E. of Pierre; Shale, Pierre Fm., Cretaceous; 65 p.p.m., b.d.
- Grass, D168; tops; young; 20 mi. S.E. of Pierre; Shale, Pierre Fm., Cretaceous; 4 p.p.m., b.d.
- Grass, D171; tops; young; 20 mi. S.E. of Pierre; Shale, Pierre Fm., Cretaceous; 34 p.p.m., a.d.
- Grass, D199; tops; young; 14 mi. N. of Bonesteel; Shale, Pierre Fm., Cretaceous; 5 p.p.m., a.d.
- Grass, D200; tops; young; 14 mi. N. of Bonesteel; Shale, Pierre Fm., Cretaceous; 4 p.p.m., b.d.
- Grass, D203a; tops; young; 14 mi. N. of Bonesteel; Shale, Pierre Fm., Cretaceous; 10 p.p.m., a.d.
- Hordeum vulgare L. (Barley), B827; grain; seed; Rumford; Shale, Pierre Fm., Cretaceous; Trace.
- Medicago sativa L., D185; tops; flower; S. of Pierre (Eldridge farm); Shale, Pierre Fm., Cretaceous; 6 p.p.m., a.d.
- Mentzelia species, D197; tops; flower; 14 mi. N. of Bonesteel; Shale, Pierre Fm., Cretaceous; Neg.
- Raphanus sativus L. (Radish), D192; Root; flower; 14 mi. N. of Bonesteel; Shale, Pierre Fm., Cretaceous; Neg.
- R. sativus L. (Radish), D192a; tops; flower; 14 mi. N. of Bonesteel; Shale, Pierre Fm., Cretaceous; Neg.
- Rosa species (Rose bush), D174; tops; young; 18 mi. S. of Pierre; Shale, Pierre Fm., Cretaceous; 3 p.p.m., a.d. Stanleya pinnata (Pursh) Britt., D571; tops; flower; 14 mi. N. of Bonesteel; Shale, Pierre Fm., Cretaceous; 299
- Triticum species (Wheat), D67; grain; seed; Fort Pierre; Shale, Pierre Fm., Cretaceous; 34 p.p.m., a.d.
- Triticum species (Wheat), D831; grain; seed; Rumford; Shale, Pierre Fm., Cretaceous; 7 p.p.m., a.d.
- Viola species, D170; tops; flower; 20 mi. S.E. of Pierre; Shale, Pierre Fm., Cretaceous; 12 p.p.m., a.d.
- Vicia linearis (Nutt.) Greene, ET4; tops; flower; 3 mi. N. of Vivian; Shale, Pierre Fm., Cretaceous; 1 p.p.m.,
- Weeds (annuals), D173; tops; flower; 20 mi. S.E. of Pierre; Shale, Pierre Fm., Cretaceous; 35 p.p.m., a.d.
- Zea mays L. (Corn), D68; grain; seed; Dixon; Shale, Pierre Fm., Cretaceous; 30 p.p.m., a.d.
- Zea mays L. (Corn) 1937, D829; grain; seed; Rumford; Shale, Pierre Fm., Cretaceous; 14 p.p.m., a.d.
- Zea mays L. (Corn) 1936, D849; grain; seed; Rumford; Shale, Pierre Fm., Cretaceous; Trace.

# TEXAS

Astragalus racemosus and Stanleya pinnata are reported to occur in the high plains country (7) of northwest Texas. Rocks of Cretaceous age (Gulf

series) occur in the Terlingua section of southwest Texas. Stanleya integrifolia, herbarium specimens (1933), mostly stems, from this section gave 525 parts per million selenium. The Gulf series of Cretaceous rocks also occur in east-central Texas from the Rio Grande River to the Red River. However, no selenium indicator plants have been reported in this area (7).

An exploratory survey was made in 1937 in the El Paso-Van Horn-Pecos section for the purpose of determining the presence or absence of seleniferous indicator plants on rocks belonging to the Comanche series. The Comanche series (lower Cretaceous) is believed to be equivalent in age to the lower Cloverly of Wyoming. No indicator plants were found in the areas traversed. Miscellaneous vegetation sampled included a wild Aster, Astragalus missouriensis, Melampodium cinereum, Nerisyrenia camporum, Streptanthus hyacinthoides, a species of mustard, and an Oxytropis. No selenium of consequence was obtained from these several specimens.

#### UTAH

Seleniferous indicator plants in Utah occur in widely separated areas. In the eastern part near Cisco, Xylorrhiza glabriuscula, X. venusta, Stanleya pinnata, and Astragalus pattersonii occur in dense growths. From this area to points above Price the seleniferous Astragali and species of Stanleya may be observed, in patches and in considerable numbers. From Cisco toward Moab, Stanleya pinnata and Astragalus preussii var. latus occur in scattered patches.

The seleniferous shale outcrop in Provo Canyon, east of the City of Provo, has been referred to earlier in this paper. The indigenous vegetation has not been examined critically.

In Box Elder County, northwestern Utah, a sample of seleniferous Astragalus toanus was obtained. In southwestern Utah a large number of seleniferous indicator plants have been collected. The important ones were Stanleya pinnata, Astragalus pattersonii, and A. praelongus.

The geological formations in eastern Utah upon which indicator plants were collected include rocks of Cretaceous and Tertiary age. The two formations of interest, in the areas explored, are Mancos shale (Cretaceous) and Green River (Tertiary). The formation in northwestern Utah was not determined in the field. The seleniferous shale in Provo Canyon is either late Mississippian or early Pennsylvanian in age. The distinctive odor, characteristic of some seleniferous plants, led Dr. David Love, U. S. Geological Survey, Field Assistant, to believe that this formation supported seleniferous vegetation. Dr. Love had previously collected seleniferous vegetation in Wyoming under our direction. Aster glaucus rooted in these shales was seleniferous. Rocks of equivalent age outcropping in other areas in Utah doubtless are seleniferous and should be capable of supporting seleniferous indicator plants.

In southwestern Utah the rocks in which indicator plants grow are believed to be Triassic (Moen-

kopi) in age.

The authors have not made explorations into the southeastern portion of Utah in the pursuit of seleniferous indicator plants. From the nature of the geological formations known to occur, it is expected that certain of the indicator plants should occur in this section of Utah.

No attempt has been made in this report to discuss the geographical distribution of the several plant genera classified by us as selenium indicators. As a matter of fact, this particular phase of the selenium problem was not considered pertinent to the original purpose of our exploratory surveys.

#### Utah Plant Samples

Aster abatus Blake, E118; tops; flower; 3 mi. E. of St. George; Shale, —, Triassic; Neg.

Astragalus species, B420; tops; past seed; 10 mi. W. of Duchesne; Shale, —, Tertiary; 2 p.p.m., a.d.

A. species, E124; tops; flower; Piute Lake; Alluvium, —, Quaternary; Neg.

A. species, E125; tops; young; 14 mi. S. of Levan; Shale, —, Tertiary; Neg.

A. species, E302; tops; seed; Kanosh; —, -—, Carboniferous?; Neg.

Astragalus aculeatus A. Nels., E543; tops; seed; Red Canyon (near Long Valley Junction); —, —, Tertiary; Neg.

A. campestris (Nutt.) Gray, E542; tops; seed; Duck
 Creek Forest Camp (near Long Valley Junction);
 —, —, Tertiary; Neg.

A. cibarius Sheld., E262; tops; flower; W. of Grantsville; —, —, Carboniferous?; Neg.

A. coltoni Jones, E249; tops; young; Monticello; Shale, Dakota?, Cretaceous; Neg.

A. confertiflorus Gray, E242; tops; flower; Price; Shale, Mancos Fm., Cretaceous; 179 p.p.m., b.d.

 A. diversifolius Gray, E247; tops; flower; Price; Shale, Mancos Fm., Cretaceous; Neg.

A. eastwoodae Jones, E525; tops; seed; 5 mi. W. of Moab; Shale, —, Triassic?; 162 p.p.m., b.d.

 A. haydenianus Gray, D563; tops; seed; 3 mi. W. of Soldier Summit; Shale, Greene River Fm., Tertiary; 22 p.p.m., a.d.

A. heliophilus (Rydb.) Tides., E263; tops; flower; W. of Grantsville; —, —, Carboniferous?; Neg.

A. lentiginosus Dougl., E117; tops; prebloom; Hurricane Junction; —, —, , —; Neg.

A. lentiginosus Dougl., E268; tops; seed; Leeds; Shale, Moenkopi, Triassic; Neg.

A. lonchocarpus Torr., E20; tops; seed; Wellington (Carbon Co.); Shale, Mancos Fm., Cretaceous; Neg.

1. lanchocarpus Torr., E20a; tops; seed; Cedar Canyon (Iron Co.); —, —, —; Neg.

 Iotiflorus Hook., E245; tops; flower; Price; Shale, Mancos Fm., Cretaceous; Neg.

A. pattersonii Gray, D557; tops; seed; 8 mi. E. of Thompson's; Shale, Mancos Fm., Cretaceous; 149 p.p.m., a.d.

A. pattersonii Gray, E119; tops; flower; St. George; Shale, Moenkopi Fm., Triassic; 313 p.p.m., a.d.

A. pattersonii Gray, E241; tops; young; Price, Shale, Mancos Fm., Cretaceous; 2154 p.p.m., b.d.

A. praelongus Sheld., E116; tops; prebloom; Entrance to Zion National Park; Shale, Moenkopi Fm., Triassic; 1284 p.p.m., b.d.

A. praelongus Sheld., E267; tops; seeds; Leeds; Shale, Moenkopi, Triassic; 178 p.p.m., b.d.

A. preussii var. latus Jones, E522; tops; seed; 3 mi. E. of Cisco; Shale, Mancos Fm., Cretaceous; 45 p.p.m., b.d.

A. pygmaeus (Nutt.) Jones, E544; tops; seed; Cedar Breaks; —, —, Tertiary; Neg.

A. toanus Jones, E15; tops; Herb. Spec.; Copper Mt., Boxelder County; —, —, —, 200 p.p.m., a.d.

A. utahensis T. & G., E254; tops; flower; Logan; —, —, Devonian?; Neg.

Atriplex species, B422; tops; seed; near Jensen; Shale, —, Cretaceous; 3 p.p.m., a.d.

Chrysothamnus species, C331; tops; seed; Crawford Mt. (near Randolph); Shale, Phosphoria Fm., Basal Triassic; Neg.

Mentzelia species, E123; tops; flower; St. George; Shale, —, Triassic; 2 p.p.m., a.d.

Oxytenia acerosa Nutt., E524; tops; seed; 10 mi. W. of Moab; Shale, —, Triassic?; 7 p.p.m., b.d.

Stanleya pinnata (Pursh) Britt., E521; tops; seed; 3½ mi. W. of State line (near Harley Dome); Shale, Mancos Fm., Cretaceous; 9 p.p.m., b.d.

S. pinnata (Pursh) Britt., E121; tops; flower; 3 mi. from St. George; Shale, Moenkopi Fm.?, Triassic; 110 p.p.m., b.d.

S. pinnata (Pursh) Britt, E122; tops; flower; 17 mi. S.E. of Zion City Junction; Shale, Moenkopi Fm., Triassic; 79 p.p.m., a.d.

S. pinnata (Pursh) Britt., E523; tops; seed; 10 mi. W. of Moab; Shale, ——, Triassie?; 3 p.p.m., b.d.

Tetradymia spinosa H. & A., E251; tops; flower; State line near Harley Dome; Shale, Mancos Fm.?, Cretaceous; 2 p.p.m., b.d.

Tetradymia inermis Nutt., E261; tops; flower; Cove Fort; Igneous, —, Tertiary; Neg.

Xylorrhiza venusta (Jones) Heller, E366; tops; seed; Cisco; Shale, Mancos Fm., Cretaceous; 14 p.p.m., a.d.

#### Bear River Bird Refuge

A preliminary study has been made of some of the prominent marsh plants of the Bear River Migratory Bird Refuge for their selenium content. The specimens were selected by staff members of the U. S. Biological Survey. Not any of the plants analyzed were found to contain toxic amounts of selenium.

The following samples, growing on Quaternary alluvium, were collected during the seed stage of growth.

Ceratophyllum demersum L., E689; tops; Neg. Chara species, E693; tops; 1 p.p.m., a.d.

Chara species, E776; tops; Neg.

Distichlis spicata (L.) Greene, E692; tops; Neg.

D. spicata (L.) Greene, E700; Basal; Neg.

Potamogeton pectinatus (L.), E694; tops; 1.5 p.p.m., a.d.

P. pectinatus (L.), E703; seeds; Neg.

P. pectinatus (L.), E778; tops; Trace.

Ruppia species, E777; tops; Neg.

Ruppia maritima (L.), E690; tops; 0.5 p.p.m., a.d.

Salicornia rubra A. Nels., E698; seeds; Neg.

Scirpus acutus Muhl., E695; rhizomes; Neg.

S. acutus Muhl., E697; seeds; Neg.

S. acutus Muhl., E702; roots; Neg.

S. paludosus A. Nels., E691; tops; Neg.

S. paludosus A. Nels., E699; seeds; Neg.

S. paludosus A. Nels., E701; roots; Neg.

Typha latifolia L., E696; roots; Neg.

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#### SUMMARY

Studies were made of 563 specimens of native plants collected from twelve western states. These studies included (1) the geographical location, (2) the rock type, the geological formation and age of the rock or soil on which the specimen was growing, (3) the stage of growth of the plant, (4) the part of the plant analyzed, and (5) the selenium concentration in parts per million. Special attention was given to species of Stanleya, Oonopsis, Xylorrhiza, and Astragalus. The Stanleyas and Astragali, because of their wide distribution, proved especially valuable as indicators. Practically all the specimens of Stanleya were seleniferous. The seleniferous species of Astragali were found to be restricted to five of the twenty-nine groups of Jones' classification of this genus.

These studies demonstrate that numerous geological formations from late Paleozoic to Quaternary in age support native seleniferous plants. These plants are rooted in both igneous and sedimentary rocks. The rocks include monzonite, limestones, and various types of shales. A number of geographical areas and geological formations not previously reported are considered.

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# THE RELATIVE HUMIDITY GRADIENT OF STOMATAL TRANSPIRATION <sup>1</sup>

Hiram F. Thut

THE GRADIENT of water vapor from the leaf and through the stomata that accounts for much of the water loss from plants is but little known and one of the least demonstrated phases of transpiration. In this gradient the rôle and value of the relative humidity of the air in the plant tissues in particular have not been demonstrated. Shaw (1935) gives measurements on the relative humidity of the intercellular spaces of some pear and apple twigs. His method of determination is based on the "turgor deficit" of cells. The turgor deficit of the cells was interpreted into terms of vapor pressure. The vapor pressure of the cells and of the air in the intercellular spaces were assumed to be the same, and this value was expressed as the relative humidity of the air. But these measurements based on the assumption that the vapor pressure of the cells and of the air in the intercellular spaces is the same does not give a true picture if transpiration is occurring through these air spaces. There will be a gradient of vapor pressure or relative humidities from the stem tissues through the intercellular spaces to the external air if stomatal or lenticular transpiration is occurring from the twigs. The vapor pressure of the intercellular spaces would thus be intermediate between that of the cells of the stem tissue and of the external air. This condition should also be true for stomatal transpiration from leaves.

The term "turgor deficit" as used by Shaw is synonymous with "suction force" of Ursprung and Blum (1916), "water absorbing power" of Thoday (1918), "suction pressure" of Stiles (1922), "suction tension" of Beck (1928), "effective (net) osmotic pressure" of Shull (1930), and "diffusion pressure deficit" of Meyer (1938). Curtis and Scofield (1933) suggested the term "turgor deficit." The term diffusion pressure deficit will be used in this paper.

Ursprung (1923), Molz (1926), Herrick (1933), and other workers have measured the diffusion pressure deficit of cells. These measurements have been made for various tissues of plants, and the plants have been under various environmental stresses. From these data diffusion pressure deficit conditions or limits of tissues are known. The vapor pressure or relative humidity of the air is a simple physical

vapor pressure gradient from leaves that is responsible for the enormous water losses through stomatal transpiration, a method should be devised that will determine the vapor pressure or relative humidity of the stomatal openings and intercellular spaces.

measurement. To complete an understanding of the

In a recent paper (1938) the author used "humidity bottles" to show the effect of relative humidity on transpiration. Data presented in that paper indicated that such bottles might be used to measure the relative humidity of the stomatal openings and intercellular spaces. For this paper such measurements were made. Small portions of leaves were exposed to various atmospheres of known relative humidity as described and illustrated in the previous paper. A brief description of a humidity bottle will be included here. The base was composed of a glass "preparation" dish having an outside diameter of 50 mm. and a height of 34 mm. Over the mouth of this dish was sealed an aluminum cap by using a rubber paraffin preparation. This cap was previously prepared by having the center raised and a hole of .95 cm. in diameter with an area of .71 sq. cm. punched through the raised portion. The area of the opening was approximately one twenty-fifth of the area of the inside of the "preparation" dish. The opening was stoppered with a tight-fitting cork stopper.

Relative humidity percentages of 100, 90, 80, 65, and 50 were maintained by using sulfuric acid solutions as worked out by Wilson (1921). In the previous work it was found that there was a tendency for the stomata to close over the sulfuric acids maintaining low relative humidities. These concentrations were not included in this series. Only sodium hydroxide pellets were used in the series to maintain a low relative humidity near 1 per cent. Potassium hydroxide was not used as formerly, because the results were similar to those of sodium hydroxide. Sufficient acid solutions or hydroxide pellets were placed in the humidity bottles to have their surfaces two cm. from the openings of the bottles.

After a series of bottles was prepared for an experiment each bottle was weighed on an analytical balance and then supported under the experimental leaf. The stopper was removed, the lower epidermis of the leaf pressed against the edges of the open-

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ing, and the leaf held in place by glass slide weights. After a period of eight hours the leaf was removed and the bottle stoppered and weighed. Variations in weight of the bottle were interpreted as water loss by the leaf to the bottle or water loss from the bottle to the leaf.

For these experiments potted plants with two definite water balance conditions were used, wellwatered plants called normal plants and wilted plants. The plants were all treated alike up to within a day or two of experimentation. The normal

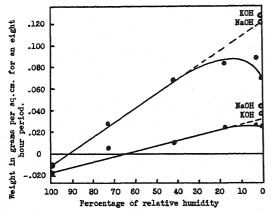


Fig. 1. Water absorption or loss for Lantana. Upper curve for normal plants and lower curve for wilted plants. Each point on the curves the average of six determinations.

or well-watered plants were then set in shallow pans so that a film of water always touched the bottoms of the clay pots. The plants designated as wilted were not watered until the degree of wiltedness was evident. By observation, by weighing the plants and pots, and by the addition of small amounts of water, these plants were held in a constant wilted condition. Most of the experiments were terminated after several days, for differences in the sizes of their leaves became evident.

The data to be presented were all collected in April—that for Lantana in 1937 and the remainder in 1938. The experiments were all performed in the same south window of the laboratory, and the plants were subjected to the temperature and humidity conditions of a classroom in nearly constant use. The temperature averaged 22°C. and the relative humidity from 40 to 50 per cent. The plants were shaded from direct sunlight by a white cloth screen. The humidity bottles were placed in contact with the leaves at 8:00 to 8:30 in the morning and were removed eight hours later. The measurements are thus for plants during that part of the day in which maximum transpiration occurs and during the time the stomata are most open. One eight-hour contact of the bottle with the leaf is designated as a determination.

The first plant used was Lantana Camara, a greenhouse plant, and the leaves experimented upon were mature. The results (fig. 1) were obtained

while data for the previously mentioned paper were being collected. The graph indicates that the water loss from the plants is inversely proportional to the relative humidity. Water losses from the normal and wilted plants are different as indicated by the two curves, the upper curve being the data for the normal plants. As most of the plant is subjected to the laboratory conditions, transpiration will cause a tension to exist in the leaf tissues. This is due to the absorption lag of the roots as explained by Kramer (1938). Those bottles maintaining high relative humidities do not absorb water from the leaf but actually lose water to the leaf because of this tension. The point on the curve where the line passes through the zero line of water loss from the leaf represents in terms of relative humidity the state of the water vapor in the intercellular spaces and stomatal openings. For Lantana the normal plants show a point of 91 per cent relative humidity, and the wilted plants show a point of 65 per cent.

These data for Lantana show the effect on the stomata of using high concentrations of sulfuric acid—i.e., in the region of low relative humidity. There was a marked closing over these concentrations which caused a definite drop in the rate of water loss. The stomata over the hydroxide pellets did not show such marked closing; a possible explanation for this effect was included in the previous paper. The data also indicate that more measurements should be made in the region of higher relative humidity. This was done on other Lantana plants, and the results verify these data.

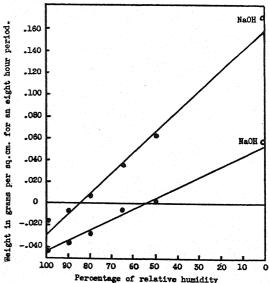


Fig. 2. Water absorption or loss for bean. Upper curve for normal plants and lower curve for milted plants. Each point on the curves the average of four determinations.

The data (fig. 2) for the kidney bean plants (*Phaseolus vulgaris*) were obtained from leaves on the first node above the cotyledons. These data obtained by the experimental series as described earlier are more regular than those for *Lantana*. The

curve for the normal plants passes through the zero line of water loss at 85 per cent relative humidity and that for the wilted at 55 per cent.

For petunia (Petunia hybrida) the data (fig. 3) were obtained from mature leaves on the sixth to tenth nodes above the cotyledons. The results were not so regular as those for bean. The normal plants have the zero point of water loss at 89 per cent and the wilted plants at 79.

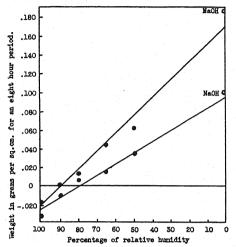


Fig. 5. Water absorption or loss for Petunia. Upper curve for normal plants and lower curve for wilted plants. Each point the average of three determinations.

The sunflower (Helianthus annuus) data (fig. 4) show the zero point of water loss from the normal plants at 94 per cent and for the wilted plants 83 per cent. The leaves on the first and second nodes above the cotyledons were used.

The data presented for these several species of plants are typical of the data obtained by using the

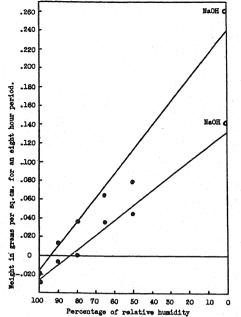


Fig. 4. Water absorption or loss for sumflower. Upper curve for normal plants and lower curve for wilted plants. Each point the average of four determinations.

humidity bottle series. Measurements were made of the diffusion pressure deficits of the several types of leaves by using the "simplified method" of Herrick (1933). Data were obtained for both normal and wilted leaves, and the average result for each set of determinations is shown in the second column of table 1. These data were obtained during the middle of the day, and leaves very similar to those used in the preceding experiments were picked for measurement. The diffusion pressure deficits of these leaves are of the same general magnitude as those

Table 1. The relative humidity gradient of stomatal transpiration as demonstrated by comparing the relative humidity of the leaf tissues, the humidity of the stomatal openings and intercellular spaces, and the humidity of the external air. All figures are averages of several determinations.

Plant and leaf condition	Diffusion pressure deficit of the leaf tissues in atmosphere	Relative humid ity equivalent of the dif- fusion pres- sure deficit of the leaf tissues	Relative humid- ity of the stom- atal openings and intercel- lular spaces	Relative humid- ity of the air about the leaf
Lantana: Normal leaf Wilted leaf		99.4% 98.6	91% 65	40–48% 40–48
Bean: Normal leaf Wilted leaf		99.7 99.5	85 55	44–50 44–50
Petunia: Normal leaf Wilted leaf		99.6 99.5	89 79	42-48 42-48
Sunflower: Normal leaf		99.4 99.2	94 83	42-50 42-50

for other plants as obtained by Shaw (1935), Herrick (1933), Molz (1926), and others. The diffusion pressure deficit values were interpreted into the equivalent relative humidities as worked out by Shaw (1935). The third column of table 1 has the relative humidity equivalents of the cells of the leaves measured.

The fourth column gives the zero points of water loss from the plants as measured by the humidity bottles. These points are being interpreted as the relative humidities of the stomatal openings and intercellular spaces because at these points the humidity of the bottles and of the air in the leaves is apparently the same. The last column gives the relative humidity variations of the air in the laboratory during the experiments as measured by the psychrometer method.

By reading across the table the relative humidity gradient of stomatal transpiration is demonstrated. A normal Lantana leaf showing a diffusion pressure deficit of 10.8 A. has a relative humidity equivalent of 99.4 per cent in its tissues. These tissues are losing water to the intercellular spaces that measure 91 per cent and the spaces to the air of the room that measures 40-48 per cent relative humidity. The wilted Lantana leaf has a relative humidity of 98.6 per cent in the tissues, 65 per cent in the intercellular spaces, and 40 to 48 per cent in the external air. The results for bean, Petunia, and sunflower are similar to those for Lantana. Thus, by this method the several large steps in the relative humidity gradient from leaves can be measured and demonstrated. The relative humidity of the intercellular spaces may be fairly low when the plant is carrying on transpiration and not at or near 100 per cent as has often been suggested.

Ramsay, Butler, and Lang (1938) made measurements of the humidity gradient at the surface of transpiring leaves. They found that a moderately transpiring leaf influenced the humidity to a distance of 2 or more cm. in slow-moving air and that as they approached the surface of the leaf with their hygrometer or evaporimeter, there was an increase in the humidity. Such a leaf caused the humidity of near 50 per cent to be increased above 60 per cent. However, for leaves carrying on low transpiration, they found it nearly impossible to demonstrate a humidity gradient. Their results are substantiated by the results from these experiments. If a moderately transpiring leaf demonstrates a partial humidity gradient outside the leaf, then the humidity of the air within the leaf should be fairly high as is true for these normal leaves. But if there is practically no humidity gradient outside a slowly transpiring leaf, then the humidity of the air within a leaf may be fairly low as is true of these wilted

These results are not without possible error. It was not possible in these experiments to measure the temperature of the leaf and of the humidity bottles. Livingston (1917), Anderson (1936), Curtis (1936), and Leighly (1937) have pointed out

the importance a few degrees of temperature may have on the relative humidities or vapor pressures. Such differences would have a marked influence on the gradient of water vapor to or from the leaves. If differences of temperature existed between the leaves and the humidity bottles, approximately the same differences should be expected through the humidity series. This would not destroy the value of showing the effect of the relative humidity on transpiration even if the whole curve as a result were shifted in position. A shift in the position of the curve would affect the zero point reading of water loss from the plants or, as interpreted, the relative humidity of the stomatal openings and intercellular spaces.

In these experiments the term relative humidity has been used rather than the more desirable term of vapor pressure because of the temperature factor. The humidity bottles maintain rather definite relative humidities but no definite vapor pressures unless the temperature is accurately controlled. These data can easily be changed to vapor pressure figures if the temperature is considered a constant at 22°C.

A second source of possible error would be in the completeness of the seal of the humidity bottle with the epidermis of the leaf. The rims of the bottles usually left a print on the epidermis or cutin, and the seals seemed vapor proof. If, however, leaks occurred, the data obtained would be lower than the actual value, for the bottles would lose water vapor to the relatively drier external air. As a result, the zero point of water loss would be a lower relative humidity figure. However, the definite and constant difference in the results from the normal and the wilted leaves would lead one to believe that if such leaks occur, their influence would be slight. Important leaks would tend to make the results from the two sets of leaves quite similar.

The opening and closing of the stomata was the one factor that might cause the most significant error in these experiments. Many of the stomata, especially of the wilted plants, would close or stay closed. The wilted condition of the leaf as well as other factors may influence the stomata. If a sufficient number of the stomata of the wilted leaves remain open so that a major portion of the transpiration is stomatal and not cuticular, then these results are significant. They show that the relative humidity of the stomatal openings and intercellular spaces, especially of wilted leaves, may be fairly low.

The data presented in this paper demonstrate the diffusion gradient of transpiration even with the consideration of possible errors. Most of all and the fact not previously demonstrated is the intermediate relative humidity of the stomatal openings and intercellular spaces when compared to the tissue losing the water and the air to which the water is lost.

### SUMMARY

The relative humidity gradient of stomatal transpiration was demonstrated by measuring the rela-

tive humidity or equivalent of the tissues of the leaves, the humidity of the stomatal openings and intercellular spaces, and the humidity of the external air. These measurements were made on normal or well-watered plants and on wilted plants. The relative humidities of the tissues were measured by the suction tension or diffusion pressure deficit method.

Measurements were made of the humidity of the stomatal openings and intercellular spaces by noting the effect various relative humidities had on transpiration. This was done by exposing small portions of mature leaves over humidity bottles that contained sulfuric acid solutions or sodium hydroxide pellets. The rest of the plant was exposed to the usual laboratory conditions. The water loss from such leaves was an inverse linear function of the relative humidity. However, in the region of high

relative humidity, water was absorbed from the bottles and not lost. The zero point of water loss from the leaves to the humidity bottles was interpreted as the relative humidity of the stomatal openings and intercellular spaces. The humidity of the air in the laboratory was measured by the psychrometer method.

A normal Lantana leaf showed a relative humidity of 99.4 per cent in the tissues, 91 per cent in the stomatal openings and intercellular spaces, and 40-48 per cent in the external air. A wilted leaf had the figures of 98.6, 65, and 40-48 per cent, respectively. Similar results were obtained for bean, Petunia, and sunflower.

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### UPWARD AND LATERAL MOVEMENT OF SALT IN CERTAIN PLANTS AS INDICATED BY RADIOACTIVE ISOTOPES OF POTASSIUM, SODIUM, AND PHOSPHORUS ABSORBED BY ROOTS <sup>1</sup>

P. R. Stout and D. R. Hoagland

THE STUDY of the movement of mineral elements in plants is greatly facilitated by the use of indicator elements which are not initially present in the plant. Bromine and rubidium have been used for this purpose by F. C. Steward<sup>2</sup> in extensive experiments on transport and accumulation. The former element has also been systematically employed in this laboratory as an indicator element. Radioactive isotopes possess certain obvious advantages in following rapid movements of mineral salts, since, first, it is possible to conduct experiments with ions which are known to be involved in the normal physiology of plants, and second, the readiness with which the radioactive isotopes can be detected in extremely small amounts enables one to make observations on small samples of tissues within relatively short periods of time.

With the cooperation of Professor E. O. Lawrence, Director of the Radiation Laboratory, J. P. Bennett of this Laboratory has made a study of the transport of radioactive potassium in the pear tree, and the present writers, also with the cooperation of Professor Lawrence, have conducted experiments on other plants with radioactive potassium, bromine, phosphorus, and sodium, as a development of an investigation of solute movement from root to shoot, especially as influenced by metabolic activities of

the root and by transpiration.

The purpose of this paper is to present experimental evidence on the path of transport of mineral elements in plants after their absorption by the roots through processes involving metabolic activities of living cells. While the prevailing view of botanists is that the mineral elements move upward in xylem tissues as the primary path of movement (cf. review by Mason and Phillis, 1937), this view has been questioned by Curtis (1935). More recently Clements and Engard (1938), following earlier work, have presented strong evidence, based on ringing experiments, in favor of the xylem as the chief path of upward movement of salt, but the problem has been raised again by Gustafson and Darken (1937) in their report on experiments with radioactive phosphate.

By using radioactive isotopes, it was possible to design experiments which, though relatively simple in their general aspect, have provided direct and definite experimental evidence of the path of movement of certain ions in well-developed, foliated plants during periods when factors of light, temperature, and humidity were favorable for plant

growth and for transpiration.

In transport experiments with radioactive phosphorus, Gustafson and Darken (1937) observed

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<sup>2</sup> Private communication.

radioactivity in all parts of the plant whether the plant had a ring of bark or a section of xylem removed. In some of their experiments, Gustafson and Darken removed a strip of bark 5 to 10 cm. long from the xylem of a geranium plant, leaving both ends of the bark attached to the wood. They found less radioactive phosphorus in the detached bark than in the bark which remained in contact with the wood and concluded that there was either an active diffusion of radioactive phosphorus from the xylem into the phloem or that injury attendant on separating the bark had interfered with the transport through it. Their general conclusion was that both xylem and phloem function in upward movement of mineral salts.

The experiments reported here are all "stripping experiments" (fig. 1a). The technique of stripping was similar to that used by Gustafson and Darken and by earlier investigators. With the aid of a Geiger counter we were able to investigate the amount of radioactivity found in a strip of bark in considerable detail and within short periods of time following the absorption by the roots of the ion under study.

If salts are shown to have moved upward in the stem of the plant, and if it is further shown that the salts are present in some tissues of the plant and absent in others, one can be fairly certain that movement did not take place through the tissues in which the salt is found to be absent. Therefore, with the stripping method a possibility should exist for obtaining a clear answer to the question of whether the path of movement is through the phloem or the xylem. The stripping operation can be performed with a minimum of injury to either of the tissues involved and should allow the normal functions of the plant to proceed with the least possible interruption. For example, Mason and Maskell (1928) found that such treatment did not hinder downward movement of sugars and that contact between bark and wood was not necessary for this transport.

EXPERIMENTAL METHOD.—The experimental plants were cotton, geranium, and willow. They were grown in culture solutions [Ca(NO<sub>3</sub>)<sub>2</sub>, .005 M; KNO<sub>3</sub>, .005 M; KH<sub>2</sub>PO<sub>4</sub>, .001 M; MgSO<sub>4</sub>, .002 M] from seedlings of cotton and from small cuttings of geranium and willow. Some geranium p'ants were also grown in sand cultures, so that there would be no possibility of mechanical disturbance of the roots. No difference was observed in the behavior of plants grown in water culture and in sand culture, with respect to salt absorption and upward transport.

A series of experiments was made on the transport of radioactive potassium, sodium, phosphate, and bromide ions. Since each individual experiment produced the same type of evidence, regardless of the species of plant and regardless of the radioactive ion used, it is sufficient for this report to offer two sets of typical data as representative of any of the experiments performed.

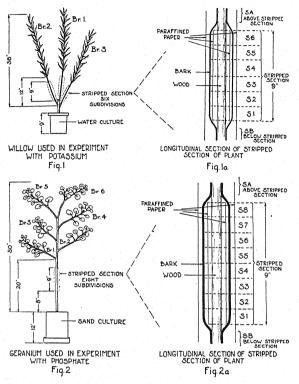


Fig. 1, 1a, 2, 2a. Illustrating the method of stripping bark and of sectioning bark and wood in experiments with willow and geranium plants made for the purpose of measuring movement of radioactive elements.

TRANSPORT OF POTASSIUM IN WILLOW (SALIX LASI-ANDRA) .- A young willow was grown from a cutting in a complete culture solution. The cutting developed 3 branches which were trained upward (fig. 1). The branches extended from the stump 36, 33, and 29 inches, respectively. Two weeks before the experiment, the branches were defoliated for a space of 12 inches above the point of attachment to the stump. Small wounds had time to heal before the bark was stripped. The leaves provided more than 50 per cent of the total weight of the branches when the plant was ready for study. At the beginning of the experiment, the roots of the plant were placed in a 2-liter beaker containing a very dilute culture solution (complete nutrient solution of approximately 0.7 atmospheres osmotic value diluted 100 times) and aerated with a sintered glass aerator. A 9-inch slit was made longitudinally in the bark on opposite sides of branches 1 and 2 (fig. 1a). The bark was then gently pulled away from the wood on each side, and a paraffined paper was inserted between the bark and wood. The entire stripped area was then wrapped with translucent paraffined paper to prevent any loss of moisture. Branch 3 was left intact, but a paraffined paper was wrapped about the outside of the branch as with the first two branches. A fan was turned toward the plant to accelerate transpiration. After an hour and a half the paper between the wood and bark on branch 2 was removed. The bark was bound to the wood with "sterilastic" tape, and the waxed paper was replaced around the outside. Neutral potassium nitrate containing radioactive potassium was then added to the culture solution in an amount to make the solution 5 milligram equivalents per liter in concentration with respect to potassium. After 3 hours of bright sunshine, followed by 2 hours of cloudy weather, the plant was removed and sectioned for analysis according to the diagram (fig. 1a).

All sections were ashed and analyzed for radioactive potassium (table 1). Quantitative technique was observed. The amounts of potassium given in the table were calculated from the measured radioactivity. When the activities were very low, the number of counts was recorded in one-minute intervals, for ten or more consecutive minutes. Only values significantly different from the background when treated statistically are given numerical expression without qualification. The lowest amounts are indicated as less than those which could be de-

tected with certainty.3

The results of the analysis given in table 1 show that after an absorption period of 5 hours, a statistically significant amount of radioactive potassium was found in all parts of the stripped branch (Branch 1, table 1) except the centrally located sections (S2 and S3) of the stripped bark. Wherever the bark was in contact with the wood (SA and SB), the concentration of radioactive potassium was as high in the bark as in the wood immediately in contact with it.

Potassium moved into the bark of Branch 2, which had the bark replaced after stripping, just as readily as it moved into the bark of Branch 3, which was left intact. This was interpreted as meaning that little injury was caused to the bark by the stripping operation.

The analyses of Branch 1 show that the bark above the stripped section (SA) had a concentration of radioactive potassium at least 150 times greater than that of the second or third (S2 and S3) sections of stripped bark. Likewise, the bark below the stripped section (SB) showed a concentration at least 250 times that of bark section S2 and S3. Further, the concentration of radioactive potassium in the wood directly opposite the stripped bark

3 The standard error of the mean of the background was determined by the formula (Snedecor, George W., Statistical Methods, Collegiate Press, Inc., Ames, Iowa, 1937):

$$s \, \overline{x} = \sqrt{\frac{Sx^2}{n \, (n-1)}}.$$

Any value of less than 3 times s x was considered as not significant of the presence of radioactivity in the tissue.

Tain of potassium in sections of willow after absorption period of 5 hours. (Calculated from radioactivity.)

	Stripped	Branch 1 Stripped 1½ hours before absorption period		Branch 2 Stripped 1½ hours, bark replaced before absorption period	replaced		Branch 3	
	P.p.m. in bark	P.p.m. in P.p.m. in Ratio	P.p.m. i	P.p.m. in P.p.m. in Ratio	P.p.m. wood	P.p.m. in	P.p.m. in P.p.m. in Ratio –	P.p.m. wood
Audyle strip SA [S6]	53 11.6				0.67 0.64	bark 64	wood 56	P.p.m. bark 0.88
Stripped section S4 S3 S2	\ \\ 5 \. \therefore \therefore \\ \therefor	112 112 160 98 5300—	155 130 132	113 89 91	0.73 0.68 0.69	87	69	0.79
Below strip SB	50 78		133 137	80 107 73	0.60   0.77   0.53	7.4	.9	000
Gain of K in leaves (p.p.m.)		h1	Тавге 1а.	Branch 2			Branch 3	16.0
Weight of leaves (gms.) Weight of branch (gms.)		21.6 37.7		8.9 16.2			9.9	
Total gammas A moved upward past 82.		870		800			18.4	

(Section S2 and S3) was at least 300 times greater than that in the bark. Since a gradient of potassium is shown from the upper bark of SA downward through the bark of S6 and S5, it is safe to assume that the potassium found in the uppermost sections (S6 and S5) of the stripped bark moved there from the bark immediately above, and not from the sections below.

PHOSPHATE TRANSPORT IN GARDEN GERANIUM (PELARGONIUM ZONALE) .- A geranium plant was grown in sand culture. At the time of the experiment, the plant was approximately 50 inches high, with 6 well-developed branches (fig. 2). The first 2 branches were 20 inches above the surface of the sand, and the stem below them was kept defoliated for a period of 2 months before the experiment. When harvested, the entire shoot was found to weigh 300 gms. Seventeen per cent of this weight was contributed by the leaves. A 9-inch strip was made 8 inches above the root crown in the same manner as previously indicated for the willow branches. During the operation of stripping the wood was scraped. After stripping, one liter of a dilute culture solution modified to include 1 milligram equivalent of KH2PO4, containing radioactive phosphorus, was added each hour to the sand culture and allowed to drain freely. During the absorption period the plant was placed outside the greenhouse. The weather was fair, with moderate wind. Six hours after the first addition of the radioactive phosphate, the plant was harvested and sectioned for analysis (fig. 2a). After washing the sand away from the roots, they were examined and found to be in good condition as judged by external appearance.

The results of the analysis (table 2) show that after an absorption period of 6 hours, significant amounts of radioactive phosphorus were found in all parts of the plant with the exception of the centrally located sections of the bark on the stripped section (S2 to S4). The concentration of radioactive phosphorus in the bark above the strip (SA)was at least 100 times that in the bark of Section S2 to S4. Similarly, the concentration of radioactive phosphorus in the bark below the strip (SB) was at least 100 times greater than the concentration in the bark of  $S\mathcal{Z}$  to  $\widecheck{S4}$ . At the same time, the concentration of radioactive phosphorus in the wood of S4was at least 300 times that of the bark of S4. As with all experiments performed, there is found a gradient in the bark from the attached ends of the stripped bark toward the central part. It is certain that the relatively high amounts of radioactive phosphorus found in the bark above the strip were brought there by lateral transfer from the wood to which it was attached and not by any upward movement of the radioactive phosphorus through the bark.

The measurements of radioactivity in the central sections of the strips of bark did not attain a level of significance (see footnote 3), and the estimates of radioactivity used for computing ratios represent

Table 2. Gain of phosphorus in sections of geranium after absorption period of 6 hours.

(Calculated as PO<sub>4</sub> from radioactivity.)

		Gan		O <sub>4</sub> presen ctions	tin	P.p.r		h wt.) PO tions	4 in
		Bark	Wood	Petioles	Leaves	Bark	Wood	Petioles	Leaves
Branch	6	197	470	191	532	12	26	44	33
Branch	5	202	360	200	790	14	24	57	62
Branch	4	151	290	55	280	16	27	37	37
Branch	3	41	62	26	94	13	17	50	49
Branch	2	175	326	86	354	18	33	48	52
Branch	1	128	286	57	117	15	32	16	19
Stem above strip	$\mathbf{S}\mathbf{A}$	270	860			16	37		
	(S8	9.0	112			4.5	44		
	S7	.5	120		• •	.28	44		
	S6	.6	132			.3	49		
Ct	S5	.8	138			.4	51		1.
Stripped section	\S4	<.3	147		••	< .16	54		
	S3	<.5	137			<.25	56		
	S2	<.3	152			<.16	.58		
	S1	11.1	131	••		6.0	41		
Stem below strip	SB	316	442	••		24	41	***	• • •
		Total gar	nmas P	$O_4$ moved	past S2=	= 7600			

<sup>\* 1</sup> gamma is equal to .001 milligram.

maximum values. Therefore, when a ratio for wood to bark of "at least 100 to 1" is reported, the actual ratio may have been much larger.

It is of interest to note that in a 4-hour absorption experiment with geranium, using radioactive sodium of about ten times the activity of the potassium or phosphorus, we found the concentration in the attached bark above the strip SA (fig. 2) to be at least 1500 times that of the stripped bark of section S3. The attached bark below the strip SB had a concentration of at least 2000 times that of the stripped bark of S3. The concentration of radioactive sodium in the wood of S3 was at least 2000 times greater than the bark of S3. This demonstrates that radioactive sodium, as well as potassium or phosphorus, found in the upper bark SA could have arrived there only through rapid lateral transfer from the xylem immediately attached. In this particular experiment, sodium to a total of 3.4 milligrams or 3400 gammas (calculated from the activity of the sample used) moved from the solution into the plant above the stripped section. Also the wood of S3 gained 76 gammas of sodium, while the bark of S3 gained less than 0.01 gamma. Thus we can state that more than 340,000 times as much sodium had moved past the stripped section into the upper parts of the plant as could be found in the bark of the stripped section S3, while at least 7600 times as much sodium was present in the wood of section S3 as in the bark of this section. It appears conclusive from these data that sodium, potassium, or phosphate must have moved upward through the xylem. If there were any upward movement of these ions through the bark, it must have been comparatively insignificant.

When the stripping technique is used, evidence for the preponderant movement of salts through the xylem becomes less sharply defined with longer periods of absorption. For example, in an absorption period of 20 hours, radioactivity could be detected in all parts of the stripped bark. Nevertheless, a downward gradient in the bark from SA to S3 and an upward gradient from SB to S3 was still demonstrated. The conclusion regarding the relative amounts of radioactive ions moving upward through the xylem and phloem would not be altered.

The observations made after longer periods of time, indicating a slow non-polar movement of small amounts of radioactive potassium, sodium, or phosphorus in the phloem would be in agreement with the evidence reported by Gustafson and Darken (1937). However, the assumption could be made that under the conditions for plant growth prevailing in their experiments, the amounts of radioactive phosphorus moved upward in their plants were extremely small, either because of low absorption by the roots or low transpiration, or both. It is conceivable that if transpiration were greatly decreased by conditions of low light intensity, high humidity, and defoliation, the upward movement of salts with water under the influence of transpiration might be decreased to such an extent that it would not differ greatly from the movement in the phloem. Under these circumstances upward movement of salt might be very slight in comparison with the amounts of salt normally moved upward through the xylem under conditions of active plant growth. The data reported by Gustafson and Darken do not permit an evaluation of the quantity of salt translocated.

Conclusions.—The following interpretations are made of these experiments, and of other experiments which will be presented in more detail elsewhere.

When salts are absorbed by roots, some portion enters the xylem within very short periods of time and is carried rapidly toward the leaves under the influence of transpiration. (In the transpiring plants used in these experiments, radioactivity could always be detected in the uppermost leaves within an hour or less after the radioactive element had been added to the culture medium.) As the salts move upward in the xylem, they are continuously transferred to surrounding living tissues. The amounts of mineral salts which move into the bark by this lateral transfer are far greater than the amounts which normally move longitudinally in the bark. As a result of all the processes of movement, there will occur a general distribution of mineral nutrients in the plant very soon after the nutrients are absorbed by the roots from the soil or other culture medium. The same type of behavior has been demonstrated by radioactive phosphate, potassium, sodium, and bromide ions.

These general conclusions are consistent with those reported by numerous other investigators who used different methods of study. They are also consistent with unpublished experiments conducted in this laboratory from other points of approach to the problem, such as the study of the effects of transpiration on movement of salt from root to shoot and of processes of exudation and guttation. We desire, however, to avoid giving any impression of over-simplification of the general question of salt movement. No attempt is made in this article to discuss the fundamental question of accumulation of salt by living cells of the shoot as influenced by the factors of cell metabolism and growth, and obviously the data presented are incapable of adding to our knowledge of the mechanism of water movement.

### SUMMARY

Radioactive isotopes of potassium, sodium, phosphorus, and bromine were used in studying the upward movement of salt in actively growing and transpiring willow and geranium plants, after absorption of these isotopes by the roots. The technique of isolating a strip of bark from the wood was followed. In this strip of bark the radioactive elements moved extremely slowly. Within short periods of time, no certainly significant amount of radioactivity could be detected in the central section of the strip while large amounts were present in the wood. However, where wood and bark were in contact, radioactive elements were rapidly transferred laterally from wood to bark. The evidence is consistent with the view that the xylem is the path of rapid upward movement of salt.

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### DIFFERENTIAL DISTRIBUTION OF ASH IN STEMS OF HERBACEOUS PLANTS FROM BASE TO TIP <sup>1</sup>

### A. E. Edgecombe

In presenting the material of plants to a class of students in botany, it is often necessary to indicate not only the various mineral elements normally present in various parts of the plant but also the quantity and distribution of these minerals in the different regions of the plant. A search of the literature revealed the surprising fact that such a group of data was almost entirely lacking to the student and instructor.

Two sources of literature on this subject were available for consultation. With plants Tukey and Green (1934) had shown for the rose, Rosa multiflora (Thurnb.), that there is an increasing ash content from base to tip of the stem on the basis of <sup>1</sup> Received for publication March 1, 1938.

both dry and wet weight calculations, as well as an increasing water content from base to tip.

The study of ash and water gradients in animals affords an interesting comparison. The studies of Kopenhaver (1937) and Van Deursen (1935) reveal that in an analysis of the body tissues of the worm, Lumbricus terrestris L., on a basis of both dry and wet weight calculations, there is basically a decreasing ash content from posterior to anterior ends of the worm and a decreasing water content from posterior to anterior ends.

Worms, therefore, appear to show a decreasing ash and a decreasing water content from the posterior to the anterior end (Van Deursen, 1935). Roses, however, appear to show an increasing ash

and an increasing water content from the base to the top of the plant (Tukey and Green, 1934). Assuming that the posterior end of the worm parallels the base of the plant and that the anterior end of the worm parallels the top of the plant, the situation then in regard to roses seems the complete reverse of the situation found with worms.

In view of this diverse relationship regarding ash and water gradients in plant and animal, and because so few data on this subject matter among plants are available, the present study was undertaken. This work, however, is limited in its scope to a study of the situation only as it applied to the herbaceous type of plants.

MATERIALS AND METHODS.—The data for these records were obtained from plants grown in the greenhouse under uniform conditions of light, temperature, humidity, and soil composition. One group of plants was grown during the months from September to December, and a second group was grown during the months from January to April.

Two kinds of tobacco plants, White Burley and Big Havana, were used; one kind of cabbage, New Jersey; two kinds of tomato, Bonny Best and John Baer; also Bryophyllum pinnatum Kurz was used to complete the series of plants used in these experiments. All the plants used for experimental purposes were grown from seed for a period of approximately four months.

At the end of the period of growth, the plants were uprooted and freed from soil. Immediately after the removal of the leaves, sections of the stem were taken from the tip, the middle, and the base of the plants. In collecting the sample of tissue from the tip of the stem, the apical sections, each one centimeter in length, taken from ten different plants were pooled to make a single composite sample. The same procedure was used in collecting the samples of tissue to represent the middle of the stem and also those to represent the base of the stem. In each of the three cases, when a sample was obtained, it was immediately weighed and its wet or fresh weight recorded.

The sample so constituted was then dried to constant weight at 100°C. in a thermostatically controlled hot-air oven. This air-dry weight was then recorded. The sample was later ashed to constant weight in a porcelain crucible, and the ash content of the sample recorded. The same procedure was followed in obtaining the wet weight, the air-dry weight, and the ash-content weight of each of the other composite samples of stem tissue—namely, the composite sample made up from ten pieces, each one centimeter long, taken from the middle of the stems, and finally the sample made from the ten pieces collected from the base of the stems.

All three composite samples of stem tissues, taken from the plants grown during the months from September to December, were carried in duplicate sets, and contrasting duplicate sets of tissues were sampled at another season of the year—namely, from the plants grown during the months from January

to April. This procedure was followed for all six varieties of plants used in these experiments.

From the weights so obtained and compiled from these experiments, the percentage ash to dry weight and the percentage ash to wet weight were calculated, and these percentages were made the basis for determining whether an increasing or a decreasing ash gradient existed in plants of the herbaceous types. Following a similar procedure of drying and weighing the selected plant portions, the percentage of water on a wet weight basis was obtained along the axis of the stem.

Table 1. The percentage by weight of the ash content (A.) of the stem to the dry weight (D.W.) and of the ash content (A.) of the stem to the wet weight (W.W.) at the different levels measured in the stem.

Stem	Base	Middle	Tip
1 Tobacco (White Burley)			
% A./D.W	5.89	10.14	12.93
% A./W.W	1.17	1.03	.89
2 Cabbage (New Jersey)			
% A./D.W	4.63	5.42	7.24
% A./W.W	1.38	1.36	1.22
3 Tobacco (Big Havana)			
% A./D.W	7.56	9.71	10.84
% A./W.W	.97	.90	.71

RESULTS AND RECORDS.—In these records only the data obtained from the series of plants grown in the autumn are used here to support the conclusions established by these experiments. While the data, secured by the experiments on the series of plants grown in the winter, are somewhat different quantitatively, they are alike qualitatively and show the same kind of increasing or decreasing ash gradient and, hence, support with equal certainty the general conclusions. Insertion of the second series of data would add nothing to the interpretation or clarification of the problem, and so, in the main, they are omitted entirely.

The results given in table I show that there is an increasing ash gradient from base to tip of the stem when measured on the basis of dry weight and that there is a decreasing ash gradient from base to tip of the stem when the measurement is made on the

Table 2. The same designation as for table 1 with different plants.

Stem	Base	Middle	Tip
1 Tomato (Bonny Best)			
% A./D.W	8.39	11.73	16.03
% A./W.W	1,29	1.11	1.08
2 Bryophyllum pinnatum			
% A./D.W	11.97	18.15	19.55
% A./W.W	1.26	1.12	.97
3 Tomato (John Baer)			
% A./D.W	8.92	12.35	16.40
% A./W.W	1.35	1.29	1.09

basis of wet weight. It was noted further that the data obtained for the second series of plants (winter-grown plants not used in these records) parallel those recorded here.

Examined in the light of the data assembled in table 1, the data recorded in table 2 show the same type of increasing or decreasing ash gradients on the basis of dry or wet weights, thus supporting and confirming the general principles and conclusions already established by the data of the former table.

Table 3. The percentage water content on a wet weight basis.

	Stem	Base	Middle	Tip
1	Tobacco (White Burley)	*87.15 86.46	*91.68 90.06	*92.65 91.17
2	Cabbage (New Jersey)	*66.72 70.38	*71.83 75.21	*83.43 83.13
3	Tobacco (Big Havana)	*87.06 85.59	*89.85 89.18	*94.10 90.11
4	Tomato (Bonny Best)	*86.66 86.99	*90.72 89.23	*91.95 91.67
5	Bryophyllum pinnatum	*89.45 87.39	*94.23 92.66	*94.88 95.04
6	Tomato (John Baer)	*84.83 88.62	*87.10 89.09	*93.34 92.83

The upper numbers of the two series of figures for each plant, starred (\*) in table 3, represent the results obtained from the experiments on plants grown in the autumn. The parallel sets of figures recorded in the lower one of the two series of figures for each plant, not starred in table 3, were obtained from the experiments on plants grown in the winter. In all instances the experimental results for herbaceous plants indicate an increasing water content from base to tip of the stem.

The experimental data assembled in table 4 show that the percentage ash content, figured on a dry weight basis, and the water content, estimated in percentage on the basis of wet weight, parallel each other in both herbaceous and rose plants (Tukey and Green, 1934), thus showing in both instances an increasing ash and water gradient from base to tip of the stem. On the basis of wet weight, however, the ash content for herbaceous plants, estimated in percentage, is reversed.

A further examination of the data summarized in table 4 shows that the percentage gradients of ash for herbaceous plants on the basis of dry weight and the percentage water content on the basis of wet weight are exactly opposite to the gradients on the same basis for worms (Van Deursen, 1935), the plant showing increasing, the worm decreasing gradients. However, the percentage gradient of ash for herbaceous plants on the basis of wet weight follows the same order as the percentage gradient of ash for worms, both showing decreasing gradients.

Discussion.—The aim of this investigation is to discover and interpret the distribution of the ash content of herbaceous plants along the stem from base to tip, when calculated on the basis of dry weight and also on the basis of wet weight. The distribution of the water content along the stem on the basis of wet weight is also included.

While work is in progress to determine, if possible, on a qualitative basis the kinds and gradient distribution of the common mineral constituents present in the ash, the experiments reported in this piece of research deal entirely with the ash content on a purely quantitative basis, and no attempt has been made to use the tentative data already obtained on calcium distribution to figure in these interpretations.

In view of adequate data obtained in this investigation and summarized in tables 1 and 2, it seems fairly evident that there is a definite ash gradient in herbaceous plants on the basis of dry weight, increasing from base to tip of the stems. These results agree in principle with the results obtained by Tukey and Green (1934) from an ash analysis of roses. They found an increasing ash gradient on the basis of dry weight in the one-year-old stems of a species of field-grown rose, Rosa multiflora (Thurnb.), that had been propagated by cuttings.

Table 4. Comparison of summary data in rose, herbaceous plant and worm. Key to symbols: A = % ash; W = % water; D.W = dry weight; W.W = wet weight; 0 = middle weight; + = increasing gradient; - = decreasing gradient.

Summary da Tukey and Rose pl	Green			ary data author aceous j				ary data an Deur Worm	
A. A. D.W. W.W	. W. . W.W.		$\frac{A}{D.W}$	A. W.W.	$\frac{W}{W.W}$ .		A. D.W.	A. W.W.	W.
+	+	Tip	+	<u> </u>	+	Anterior	-		
0 0		Middle			•	M(3.11	•••	•••	• • •
	•	Middle				Middle	0	0	
		Base	-	+	<u></u>	Posterior	+	+	+

The data recorded in tables 1 and 2 show, on the other hand, that on the basis of wet weight there is a decreasing ash gradient from base to the tip of the stem in herbaceous plants. This finding differs from that obtained by Tukey and Green (1934) in their work. They found for roses an increasing ash gradient, calculated on the basis of wet weight. This finding paralleled the increasing ash gradient that they found for roses on the basis of dry weight.

As is indicated in table 3, there is sufficient evidence of a confirmatory nature to support the conclusion that in herbaceous plants there is an increasing water content from base to tip of the stem, calculated in percentage on a wet weight basis. A parallel increasing water gradient is shown by Tukey and Green (1934) for the stems of rose plants. The increasing water content appears to be due in some way to the water associated with the greater amount of mobile protoplasm in the living tissues that increases from base to tip of the stem in herbaceous plants.

The data summarized from the thesis of Van Deursen (1935) are compared with the summary data found by the writer through experimental investigations on herbaceous plants. The comparative data are assembled in table 4 and represented by "plus," "minus," and "zero" symbols. The data show for worms a uniformly decreasing gradient for ash on the basis of body dry-weight, also a decreasing gradient for ash and water on the basis of body wet-weight, when calculated on a percentage basis from posterior to anterior parts of the worm.

These data seem to indicate, in general, a reversal of gradient for ash and water content in worms, measured from posterior to anterior ends on a percentage basis as compared to the gradients for ash and water on the basis of dry and wet weight in the stems of herbaceous plants, where in two instances there are increasing gradients. However, considering that the growing region of the worm is toward the posterior end, it is then not so surprising to find an increasing ash and water content appearing in that direction, particularly so since the metabolic gradient in the worm progresses from the anterior toward the posterior end. If, therefore, the metabolic gradient is from base to tip in the plant, the growing region in those plants examined being toward the tip, then the reversal for ash and water content in worm and plant is not a contradictory phenomenon but a similar behavior, since in each instance the increasing ash and water content is correlated with and parallels the metabolic gradient and also increases in the direction of growth for both plants and worms.

The data for rose plants, abstracted from the article by Tukey and Green (1934) are contrasted with the summarized data of the writer for herbaceaus plants and show for roses a uniformly increasing gradient for ash on the basis of dry weight and also for ash and water on the basis of wet weight. These data seem to indicate, in the main, that the gradient values for herbaceous plants, as is

indicated in table 4, follow a parallel course to that of rose plants. Only in the case giving the ash value on the basis of the wet weight is there a decreasing gradient for herbaceous plants.

In general, then, it appears that the increasing ash content for herbaceous plants, from the base to the tip of the stem, is due, in part, to a greater accumulation of such materials as carbohydrates and fats at the base than at the tip of the stem, which materials contain less minerals, and in part, at least, to an increased accumulation of protoplasm toward the tip of the stem apparently accounting for the increase of ash content in that region.

### SUMMARY

Six different species of herbaceous plants, developed from seeds, were used in this investigation. These plants were grown under uniform conditions for four months in the greenhouse. Spring and winter groups of plants were examined separately for comparisons.

All tests were made in duplicate, and to preserve uniformity of tissue throughout, composite samples of plant materials, taken from specific regions of the stem, were used in the experiments.

The wet or fresh weight was taken on an ordinary balance at the time the stem tissue was collected. The dry weight at 100°C. and the ash weight of the tissue after incineration were taken on precision balances. The water content was calculated from the wet and dry weights.

When samples of stem tissue were taken from the base to the tip of the plant, an increasing ash gradient on the basis of dry weight, a decreasing ash gradient on the basis of wet weight, and an increasing water gradient on the basis of wet weight were uniformly obtained.

In general the ash and water gradients, determined for herbaceous plants, are the reverse of those found for worms, except for the ash content on the basis of wet weight where there seems to be a parallel situation, or similarity of findings for both plant and worm.

The ash content on the basis of dry weight and the water content on the basis of wet weight, however, for both plant and worm, are correlated with the metabolic gradient and parallel the direction of growth in these organisms.

In the main, the ash and water gradients determined for herbaceous plants are of the same order and direction as those found for rose plants, except for the ash gradient on the basis of wet weight, where there seems to be a dissimilarity of findings for herbaceous and rose plants.

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### THE RELATIVE ACTIVITIES OF DIFFERENT AUXINS 1

Kenneth V. Thimann and Charles L. Schneider

Now that so many substances have been found to behave as auxins in promoting the growth of plants, it becomes of increasing importance to be able to compare their activities with some precision. This is particularly necessary in any study of the relation between structure and activity. It has been pointed out earlier (Koepfli, Thimann, and Went, 1938) that the mere presence or absence of growthpromoting activity is sufficient criterion for a first attack on the problem, but the quantitative comparison of activities introduces additional complexities. This is in part due to the fact, first elucidated by Thimann (1935), that the relative activity of any substance, compared to another substance as standard, varies according to the method of assay. Thus, even if the most fundamental physiological property of auxins-namely, growth promotionwere used, Thimann found that indene-acetic acid had only 1 per cent of the activity of indole-acetic acid by the curvature method, but about 7 per cent by straight growth. The test object was the Avena coleoptile in each case. When tests based on root initiation or bud inhibition were compared, the influence of the method of assay was still more marked. Other examples of the same principle may be seen in the table of Went and Thimann (1937, p. 137) which summarizes the activities of numerous auxins found by various investigators with different meth-

We have recently had occasion to compare the activities of some pure auxins and related compounds on straight growth and on curvature, using the same test plant and also different plants. The results and conclusions form the subject of this note.

Materials and methods.—The following seven compounds were studied: indole-3-acetic, -propionic, and -butyric acids; benzofurane-3-acetic, 2 alphanaphthalene-acetic, 3 phenyl-acetic, and gammaphenyl-butyric acids. Of these compounds, only the indole and naphthalene derivatives give definite auxin curvatures in the standard Avena test (see table XII of Went and Thimann, 1937), though the other substances may give small curvatures, both positive and negative, at longer intervals after application.

The methods used were (1) direct measurement of elongation of isolated sections of Avena coleoptiles and of Pisum stems (method of Bonner, 1933,

1 Received for publication March 4, 1938.

<sup>2</sup> Synthesized by Titoff, Muller, and Reichstein (1937). <sup>3</sup> Sample of high purity prepared by Dr. Franklin Jones, Chemical Paint Company. with modifications of Schneider, 1938) and (2) curvatures of slit *Pisum* stems (method of Went, 1934, with modifications of Thimann and Schneider, 1938). The coleoptiles were used at an age of 75–77 hours from soaking. The peas were aged about eight days, the third internodes used being then well developed but still growing. Three sections each 3 mm. long were cut from each plant, beginning at 5 mm. below the tip in *Avena* and about 10 mm. in *Pisum*. With *Avena*, not less than 10 plants—i.e., 30 sections—were used for each solution tested. With *Pisum*, 15–25 sections were found sufficient.

For the slit stem curvature, about 1 cm. was cut off the tips and the zone below it halved longitudinally for a distance of 3 cm. Unless otherwise stated, the sections and slit stems were immersed in shallow layers of the test solutions directly after cutting.

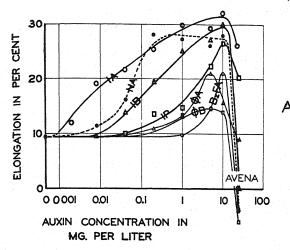
The plants were grown and tests carried out in a physiological darkroom at 24°C. and 85 per cent relative humidity with only occasional red light. Under these conditions, by far the largest part of the elongation and curvature of the tests was completed in 24 hours. Measurements were made at 30–60 hours. For the sake of completeness, some curvature measurements in the *Avena* test are also included.

RESULTS AND CONCLUSIONS.—Figure 1A shows the activities of the different auxins as determined by straight growth of sections of Avena coleoptiles. It represents the average of two separate experiments performed on different dates but showing close agreement. Each experiment involved 38 solutions and a total of more than 1100 coleoptile sections. A third experiment, which did not include as many concentrations, is incorporated into the points at 1 and 5 mg. per liter and also gives the points at 25 mg. per liter. The concentrations around the optimum were studied again in a separate experiment. These data are plotted on an arithmetical scale in figure 1B. From both figures it is clear that the optimum concentration for each auxin is not far from 10 mg. per liter. It will be noted that indoleacetic acid exerts growth-promoting activity at a much lower concentration than any other substance and that the curves for the three-indole acids are well separated.

Figure 2A, also the average of two complete experiments, shows similar results for the straight growth of *Pisum* stem sections. Comparison of the two figures shows that for all auxins, except per-

haps indole-propionic acid, Avena shows detectable increase in growth at lower concentrations than Pisum. Similarly the optimal concentrations are on the whole lower for Avena than for Pisum, for in the latter definite toxicity does not appear up to 25 mg. per liter (see also fig. 7 of Thimann and Schneider, 1938). The whole range of effective concentrations in Avena is thus from two to five times as low as in Pisum.

With regard to relative activities, it is clear that the auxins fall in about the same order in both cases,



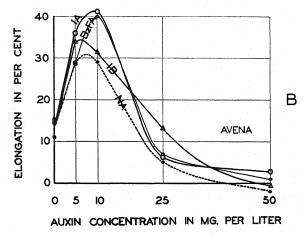


Fig. 1.—A (above). Elongation, as percentage of their initial length, of 3 mm. sections of *Avena* coleoptiles in solutions of different auxins. Lettering of curves (as in all other figures):

IA, indole-3-acetic,IB, indole-3-butyric,

NA, alpha-naphthalene-acetic,

IP, indole-3-propionic,

 $\phi A$ , phenyl-acetic

 $\phi B$ , gamma-phenyl-butyric,

BzFA, benzofurane-3-acetic acid.

Mean of two complete series of curves (Apr. 21 and May 3, 1938).—B. Another experiment, showing only higher concentrations to indicate the optima. Note that the scale here is arithmetical (Feb. 9, 1939).

but there is a considerable quantitative difference between the two plants. The curves for the indole acids are much more widely separated for Avena than for Pisum. On Avena, indole-acetic is about ten times as active as indole-butyric and 100 times

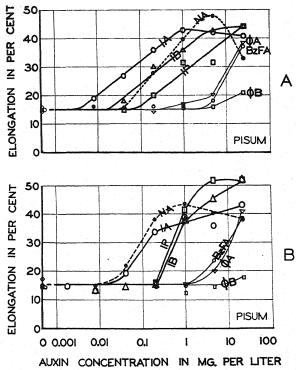


Fig. 2. Elongation, as percentage of their initial length, of 3 mm. sections of etiolated *Pisum* internodes.—A (above). Mean of two series of curves (Mar. 28 and Apr. 13, 1938).—B. Experiment of May 4, 1938. The values for the four sets of water controls are plotted separately to give an indication of their close agreement. Same auxins as figure 1.

as active as indole-propionic, while on Pisum the corresponding values are about four times and sixteen times, respectively. It is interesting to note that the activity in straight growth alternates with increasing length of the side-chain, in the same way as has been found for the standard Avena test (Went and Thimann, 1937, p. 137). Further, phenylbutyric acid shows activity at 1 mg. per liter on Avena but not below 10 mg. per liter on Pisum. In one or two of our experiments, phenyl-butyric acid showed no activity at all on Pisum. The relation between the curves for a-naphthalene-acetic acid and indole-acetic acid is the same as that reported by Scheer (1937) in straight growth measurements of Avena, made by symmetrical application of agar blocks for eight hours. Her curve for naphthaleneacetic acid is lower than that for indole-acetic acid at low concentrations, but the curves cross at 0.3 mg. per liter. Ours cross at 0.1 mg. per liter (fig. 1A). In general it is clear that not only absolute activities but the relative activities of the different auxins, compared to indole-acetic acid as standard,

depend on the test plant used.

Relative activities also depend upon some factor more difficult to define. Figure 2B shows another series of experiments on the elongation of Pisum sections, using the same auxins. The curves differ considerably from those of figure 2A. The minimal concentrations giving increased growth are higher and the slopes of the curves in the zones where growth is a function of auxin concentration are much greater. In addition, the relations of the auxins to one another are considerably changed. The curves for indole-butyric and -propionic acids, instead of being well separated, almost coincide, while indoleacetic acid is less active than naphthalene-acetic acid over the whole range. Two considerations show that these variations are real: (1) each point is the mean of 15-21 sections, the range of distribution of whose individual growth values is far less than the difference between two corresponding points, and (2) the points on each curve support one another, so that the differences are between whole curves and not just individual points. Of the four complete experiments performed, two gave results which agreed well and are averaged in figure 2A, one gave the data of figure 2B, and the other gave results divergent from both, but somewhat resembling those of figure 2B. The differences could not be correlated with any observable differences either in the plants used or in the lengths of the internodes at the time they were taken. Similar differences in relative activities on different days have been found with straight growth of Avena coleoptile sections.\* Such variations are fairly large, and, as may be seen, introduce both quantitative and qualitative differences. The causes are obscure but must comprise some superficially minor differences in the conditions or the treatment of the plants. It is evident, however, that the relative activities of different auxins vary not only with the plants used, but also with their sensitivity on the day of the test.

Figure 3 shows the results of three complete experiments (A and B) on slit stem curvatures ("pea test" of Went, 1934). Each involved about 250 individual curvatures. Measurements are by the "stem reference" method (see Thimann and Schneider, 1938). The most obvious difference between these data and those above on straight growth is that here there is little consistent difference between the activities of the four most active auxins, supporting the statement previously made about this test that "indole-3-acetic, indole-3-propionic, indole-3-butyric, and alpha-naphthalene-acetic acids at low concentrations approach the same activity per mol" (Went and Thimann, 1937, p. 135). If one takes the average of all the data, the differences between the

\*The same phenomenon occurs even in the standard Avena curvature test. The relative activity of indole-butyric acid, determined by this test, varied on different days between the limits of I per cent and 15 per cent of that of indole-acetic acid.

activities of the three indole acids, which are so marked in figure 1, practically disappear.

It has been shown further by D. Bonner (1938) that if correction is made for differences in dissociation constants, the activities of a number of auxins, but not all, approach one another closely. It is clear from figure 3, however, that in individual experiments the activities of different substances are in no case truly identical. The same evidently holds for figure 6 of D. Bonner (1938).

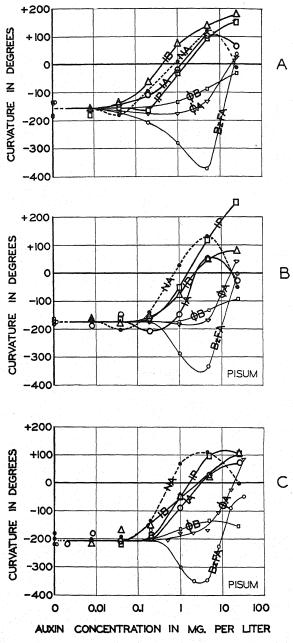


Fig. 3. Three measurements of auxin activity by means of slit stem curvatures with *Pisum*. Same auxins as figure 1 (Mar. 28, Apr. 14, and May 4, 1938).

Table 1. Concentrations of different auxins giving minimal and maximal responses of straight growth and of slit stem curvatures with Pisum.

	Conc	entration in m	g. per liter gi	ving:
	Just detect- able straight growth	Just detect- able inward (or decreased outward) curvature	Maximum straight growth	Maximum inward curvature
Indole-acetic (IA)           Indole-propionic (IP)           Indole-butyric (IB)           Naphthalene-acetic (NA)           Phenyl-acetic (\$\phi\$A)           Benzofurane-acetic (BzFA)		0.35 0.23 0.10 0.12 3	14 20 11 4 25 25	12 20 25 5 25 25

Comparison of the absolute concentrations in figures 2 and 3 brings out a second point—namely, that each auxin (except perhaps phenyl-butyric acid) causes increase in growth at concentrations below those which cause inward curvature (see table 1). Phenyl-butyric acid behaves exceptionally on certain days, however (see below). In contrast, the concentrations which cause maximal straight growth agree very well with those causing maximal curvature.

It is perhaps worth noting that indole-acetic and naphthalene-acetic acids, in which the differences between the two threshold concentrations are greatest, are the two which give outward curvatures most frequently and regularly.<sup>4</sup> Their behavior comes nearest to that of benzofurane-acetic acid (see below) in this respect.

These facts are readily explained by consideration of the nature of curvature and of growth. Inward curvature occurs when the growth of the outer layers exceeds that of the inner (Jost and Reiss, 1936; van Overbeek and Went, 1937). However, as we have previously shown (Thimann and Schneider, 1938) for indole-acetic and benzofurane-acetic acids, in the lowest auxin concentrations it is the innermost layers which respond more than the outer. Nevertheless, the maximum increase in growth of which the inner layers are capable is small, and hence at higher concentrations the outer layers grow more than the inner, causing inward curvature. Before this concentration is reached there is an intervening range in which the growth rates of both tissues are increased. In this range, therefore, the stem shows increase in straight growth. Thus straight growth will in general be increased at concentrations lower than those which cause inward curvature.

The case of benzofurane-3-acetic acid is rather exceptional in that, as we have previously shown (Thimann and Schneider, 1938), it promotes growth of the inner layers at concentrations very much

<sup>4</sup> The production of increased outward curvatures by auxin concentrations too low to cause inward curvature was described in the preceding paper (Thimann and Schneider, 1938).

lower than those which affect the outer layers. However, there is no reason to believe that growth of a single tissue or layer suffices to cause marked growth of the whole stem. Since with benzofurane-acetic acid only the growth of the inner layer is promoted by low concentrations, marked straight growth does not begin until the growth of the outer layers is increased. This will be where the curve (fig. 3) reaches its lowest point and begins to turn upward, or at about 2–5 mg. per liter (cf. table 10 of Thimann and Schneider, 1938).

Special consideration must also be given to the action of y-phenyl-butyric acid. This substance, scarcely an auxin in the strict sense of the word, since it is almost inactive in the standard Avena test, has in some tests shown a reduction in outward curvature at concentrations lower than the minimum for straight growth. Of a total of 10 complete experiments, each with serial dilutions, either of the acid or its Na salt, curvature was shown at the same concentration as growth in 5, at lower concentration in 4, and at higher concentration only in 1. When curvature is influenced by a concentration which produces no growth, explanation is difficult. It might be suggested that the effect is the reverse of that of benzofurane-acetic acid-namely, that the outer layers respond to lower concentrations of phenyl-butyric than do the inner layers. This, however, is improbable under normal conditions, because, owing to the tension of the outer layers, any increase of their length should allow a corresponding elongation of the inner layers and hence straight growth. A second possibility—namely, that the growth of the inner layers is actually inhibited—was ruled out by direct measurement of the elongation of isolated layers of tissue and also of the inner and outer sides of the slit stem. Neither of these measurements showed any inhibition of the growth of the inner tissues at concentrations which give slight or definite inward curvature. It is worth noting that the difference between the growth of the two sides of the slit stem (o-i in fig. 4) was found to be linearly proportional to the curvature, and the proportionality was the same for phenyl-butyric as for indole-acetic acid.

The difficulties of reaching a conclusion are increased by the small magnitude of the effects of phenyl-butyric acid. Where growth is small and experimental error, therefore, large, curvature is a more sensitive response than straight growth measurement. On days when the growth and curvature

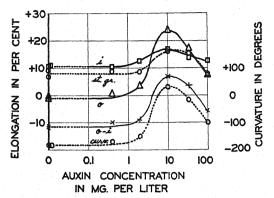


Fig. 4. Gamma-phenyl-butyric acid. Direct measurements of length of the inside (i) and outside (o) of slit halves of *Pisum* stems compared with independent measurements of straight growth (sb. gr.) and of curvature. Mean of two complete experiments (Aug. 24 and Dec. 3, 1938).

are relatively large there is excellent agreement between the two, as shown in figure 4. It is, therefore, very doubtful whether these apparent anomalies have any real significance.

Thus, for all the auxins studied, the essential characters of these curves fit in with the concepts we have previously developed and provide additional support for them. The recent experiments of Jost (1938) also confirm completely our explanation of the inward curvatures.

The three experiments of figure 3 give a sample of the variation found between different slit stem tests.

Since it is possible that the amount of auxin still in the stem after cutting influences the response to applied auxin, some tests were carried out in which the sections were cut off, placed in water for four or even eleven hours, and then transferred to auxin. This treatment, however, merely reduced the absolute curvatures somewhat. Even phenyl-butyric acid, which is practically inactive in the Avena test, still gave inward curvatures on slit stems that had been kept eleven hours in water.

It is an interesting corollary that the difference in sensitivity to auxin of two layers of tissue within the same plant is itself comparable to the differences in the sensitivity of two different plants. Thus the Avena coleoptile is sensitive to lower concentra-

<sup>5</sup> In figure 4 the straight growth is the average of four 5 mm. sections from each plant—i.e., the same zone of about 20 mm. over which the "inside" and the "outside" measurements were made. This results in a lower growth response than if three 3 mm. sections had been used as above; thus the water controls grow only 8 per cent as against 15 per cent in figure 2.

tions than Pisum but gives less absolute elongation—i.e., it behaves somewhat like the inner layers of Pisum.

Although the elongation of Avena coleoptile sections is increased by sugar, this is not as a rule true for Pisum sections, as we have previously found. This suggests that the differences in the behavior of Pisum and Avena might be due to differences in carbohydrate supply. Straight growth measurements were therefore made on Avena in presence and in absence of sugar. Although the total growth was increased throughout, the positions of the curves for the different auxins (the three indole-acids and benzofurane-acetic acid were used) remained the same and the relative activities were almost unchanged. The carbohydrate supply, therefore, is not the important factor responsible for the differences between the two test plants.

VALUES FOR RELATIVE ACTIVITIES.—It follows from the above that relative activities of different auxins vary with the test plant, test method, and the sensitivity.6 These facts make it very difficult to assess relative activities quantitatively. In table 2 the averages of our observations with the different test methods are summarized. All are expressed as percentages of the activity of indole-acetic acid. The activity for straight growth is determined by the highest dilution which produces 50 per cent greater elongation than that of the water control. That for slit stem curvatures is similarly determined by the highest dilution which decreases the outward curvature by 100° when measured by the "stem reference" method. (These values are in good agreement with those determined from the dilutions which give a curvature of 50° by the "inflection reference" method.) These dilutions (or reciprocal concentrations) are then expressed as percentage of that dilution of indole-acetic acid which has the same effect. The values for curvature in the standard Avena test are in part new determinations, in which several times of application of agar blocks, from 90 to 150 minutes, were used, and are in part taken from Went and Thimann (1937).

The important points to be noticed in the table are: (1) the numerical values vary widely with the test method, (2) the order in which the auxins fall is the same for the two straight growth methods, (3) relative activities are, throughout, lower for Avena straight growth than for Pisum straight growth—i.e., the Avena coleoptile is the more "specific" (i.e., discriminating) object, and (4) as has been made clear by the earlier work, the standard Avena test using agar is much more specific still.

It is evident that such differences as those above amply account for the discrepancies between rela-

<sup>6</sup> Van Overbeek (1938) makes the following statement: "An auxin amount expressed in hetero-auxin equivalents is independent of the test method, test plant and sensitivity." This, it will be clear, is not borne out by our experiments, nor, apparently, by the experience of Ferman in Utrecht (see Ferman, 1938).

TABLE	2. Relative	activities of	six	auxins	compared	to	the	activity of	of .
		indole-3-ace							٠.

	Curvature of slit stems of Pisum	Straight growth of Pisum sections	Straight growth of Avena sections	Curvature in standard Avena test (figures in brackets denote number of tests)
Indole-3-acetic acid	100	100	100	100
α-Naphthalene-acetic acid	370	23	15	2.5ª
Indole-3-butyric acid	190	22	9	8 (35)
Indole-3-propionic acid	150	8	1.6	0.1*
Phenyl-acetic acid	10	0.4	0.3	0.02ª
Benzofurane-3-acetic acid		0.3	0.11	0.02 (13)
γ-Phenyl-butyric acid	ca. 3	0.08	ca. 0.06	0.005 (4)

a Data from Went and Thimann.

tive activities found by different workers. Differences in the purity of samples used may also account for some of the discrepancies which have been reported. Further, when one considers that the variations described here are found with uniform plants grown and used under carefully controlled conditions, it is clear that data obtained with less critical material, such as with green plants under greenhouse conditions, have little quantitative significance.

The fact that each auxin has its own characteristic curve in the different tests could conceivably be turned to advantage for the identification of an unknown auxin. Both the shape of the curve and the relative activity in different tests could be used as identifiable characters.

### SUMMARY

Although it is well known that different substances have different activities as auxins, it is often

thought that the ratio between the activities of any two substances would always be the same. In this paper the activities of six different pure auxins in promoting growth were compared with that of indole-3-acetic acid as standard. The ratio between the activity as determined by any method and that of indole-acetic acid in the same method is termed the "relative activity." Relative activities for straight growth as determined on Avena coleoptiles differ from those determined on Pisum internodes. The differences between these two plants are not due to differences in carbohydrate supply. With Pisum the values for straight growth differ from those for slit stem curvatures. This is explained on the basis of differential sensitivities of tissues within the plant. With any of the methods, the values also vary somewhat with the sensitivity of the plants on the day of the test. By taking the mean of several series of experiments with each method, approximate relative activities have been derived and tabulated.

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### THE EFFECT OF DEHYDRATION ON MICROSPOROGENESIS IN TRADESCANTIA $^{\scriptscriptstyle 1}$

### Norman Giles

Numerous studies have been made of the effect of various agencies such as chemicals, abnormal temperature, radiation, and genetic factors in producing abnormal nuclear and cell division. Experimental observations have been made on both living and fixed material. In general it is found that many distinctly different agents produce similar effects, of which the majority seem to result from a disturbance of the normal synchronization of the cell's activities during the division cycle. Reviews of the experimental study of nuclear and cell division include those of Politzer (1934) and Küster (1935).

Recently the importance of an adequate cellular water content for the normal course of somatic nuclear and cell division has been demonstrated by the work of Wada (1936a, 1936b) on stamen hairs and Shigenaga (1937) on petal cells of Tradescantia L., and by Milovidov (1938) working on root tips of Vicia faba L. Oehlker and his students, in a series of papers dealing with studies in the physiology of meiosis, have investigated the effect of dehydration, occasionally in combination with high and low temperatures, as indicated by variations in chiasma frequency at diakinesis in Vicia faba, Campanula percifolia L. (Straub, 1937), and various species of Oenothera L. (Kisch, 1937). These studies indicate that the degree of hydration of the cell is a controlling factor in the normal synchronization of chromosome behavior and nuclear and cell division. The present investigations deal with the effect of dehydration upon cellular phenomena during microsporogenesis in Tradescantia.

MATERIALS AND METHODS.—During the course of an investigation of the effect of certain chemicals such as saponin and colchicine on meiosis in Tradescantia, it was noted that the control stems with paraffin sealed ends showed the same abnormalities as the chemically treated stems. This observation led to a more detailed study of the effects of water deficiency. Plants of a clonal line of a hybrid derived from Tradescantia canaliculata Raf. and a clonal line of T. paludosa Anders. & Woods. were used. Flowering stalks were cut and the ends sealed and coated with warm paraffin to a distance of about one cm. up the stalk. These stalks were then placed in water maintained at a level of about three cm. in containers kept in the greenhouse. In some cases the paraffin was removed from the stalks after a certain time and the free ends left in the water. In others the paraffin was left intact for the duration of the experiment. In the latter cases evidently the stems were often able to obtain some water, for some stalks

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The writer wishes to acknowledge his sincere appreciation to Prof. Karl Sax for suggestions and criticisms during the course of this work.

developed roots which broke through the paraffin. Obviously the methods used did not give any definite or uniform indication of the amount of water received by the stem. However, this was not an essential factor, since the aim was simply to study the effects of a general dehydration on meiosis and related phenomena. A few observations were also made on entire potted plants which were not watered for several days. As controls, cut stalks with unsealed ends were placed in containers of water and kept in the greenhouse. In no cases were abnormalities observed in these. Acetocarmine smear preparations of the anthers were made at intervals in order to study the effect of the restricted water supply upon meiosis and the microspore division.

Observations.—Nuclear division.—Apparently the effects of dehydration on nuclear division in Tradescantia are similar to those produced by temperature (Sax, 1937) and colchicine treatment (Walker, 1938). The restriction of the water supply results in the suppression of the spindle mechanism at the first or second meiotic division, thus preventing the normally paired chromosomes from passing to the poles. Apparently the chromosomes then reassemble into a single nucleus and proceed to divide equationally to produce dyads with the diploid number of 12 instead of the haploid number of 6. In some cases both nuclear divisions are inhibited, the 12 chromosomes resulting from the first equational division then dividing again equationally, thus producing 24 univalents organized into a tetraploid. The suppression of either the first or the second meiotic division results in the production of two diploid microspores which undergo normal mitosis at the microspore division (fig. 3, 4). The tetraploid monads also divide normally at the microspore mitosis (fig. 6, 7).

The cytological effects of sealing off the stems were first noted after 11 days. The cells were then in the young microspore stage just after the second meiotic division. The experiments were carried out in February and March, at which season the duration of the meiotic cycle is increased from the normal summer period of about a week (Sax and Edmonds, 1933) to one of about two weeks' duration (from early prophase to the stage when the young microspores begin development). These time relations seem to indicate that the interruption of the water uptake was not immediately effective in upsetting nuclear divisions already in progress at the beginning of the treatment. Apparently a certain degree of dehydration of the cells is necessary before spindle inhibition and other abnormalities result. The indications are that only those cells that were in the early stages of meiotic prophase or earlier at the start of the treatment were affected at the time of the first meiotic division.

There seems to be no constancy in the effect of dehydration in any single anther or group of anthers. The percentage of abnormalities may run as high as 75 per cent in one anther, while in another anther of the same flower there may be no irregularities at all. Haploid, diploid, and other polyploid microspores may occur in the same anther, but the normal haploid spores are almost always the most abundant. The monads are also less common than the dyads.

In one anther several giant pollen grains were observed 22 days after the paraffin treatment (fig. 5). Since none of these were seen at microspore division, no chromosome counts were obtained. However, the size of these grains as compared with that of the tetraploids indicates that their number is probably 48 or octoploid. It appears that pollen of this type results from chromosome doubling through spindle inhibition in premeiotic cells followed by two chromosomal but no nuclear divisions at meiosis as is the case when monads are produced. It has also been found that such octoploid cells are produced in *Tradescantia* by colchicine treatment (Walker, 1938).

Obviously the effect of dehydration in inhibiting nuclear division by suppressing the spindle is reversible as the chromosomes can reassemble into a polyploid nucleus which goes through succeeding divisions in a normal manner. In cases where dehydration was very intense the entire stalk was badly withered and pollen failed to develop. However, on the stems where the paraffin seal was removed, or where it was broken by the penetration of roots, mature diploid and tetraploid grains developed. Here also the later meiotic divisions, following the resumption of water uptake, showed no irregularities. The potted plants which were not watered for several days showed the same abnormalities, whereas after watering was resumed only normal divisions were found. The observations on these plants which were subjected to drought conditions suggest quite definitely that it is the disturbed water relations which are the cause of the abnormalities described.

Chromosome behavior.—As has been noted before (Sax, 1937), the microspores with the larger chromosome number are larger and develop more slowly. This decrease in the rate of development is apparently different in degree for the cytoplasm as compared with the chromosomes, for when the tetraploid microspore reaches the stage for cell division the chromosomes have shortened by spiralization until they are approximately half their normal length as seen in the haploid microspore (fig. 1, 6).

An increased shortening of the chromosomes in tetraploid as compared with diploid Tradescantias has been reported at meiosis by Sax and Sax (1937). The number of major coils is reported as 5.5 for the diploid form and 4.5 for the tetraploid. These

differences are attributed to the slower development of the larger cells of the tetraploid form. Comparable measurements are not given for diploid and tetraploid microspore chromosomes, but it is stated that those of the tetraploids are shorter (Anderson and Sax, 1936). In the present case the amount of shortening is much greater than previously reported. It was found that increased shortening of the chromosomes also occurred in the haploid microspore when the stems were cut and left to wilt for 24 hours. The chromosomes contracted until they were about two-thirds the length of the normal haploid microspore chromosomes (fig. 1, 2). Apparently the water deficiency itself in some manner causes increased chromosome contraction. It seems quite possible that the desiccation may cause a slowing down of cell development, thus permitting increased chromosome contraction by coiling. A similar condition occurs in microspores of colchicine treated plants, where contracted metaphase chromosomes are observed (Walker, 1938). In the microspores observed, the dehydration was not sufficient to interfere with the spindle mechanism. In fact, in no case was an inhibition of the mitotic division to form the two microspore nuclei observed. This division seems to be less easily upset than those of meiosis.

Cell division.—Besides inhibiting nuclear division, dehydration caused striking deviations in the normal rhythm of cell and nuclear division. In many cases cell division was entirely inhibited after nuclear division had occurred, resulting in bi- or quadrinucleate cells. In other cases, cell division occurred without nuclear division, occasionally in such a manner as to produce one nucleated and one enucleated cell. In some cases cytokinesis appears to be initiated but is later arrested. The division may be very unequal and a small mass is cut off from the side of the cell (fig. 9). The cell wall may constrict the nucleus, but actual bisection has not been observed (fig. 8). These abnormal cells may continue development and produce pollen grains of bizarre shapes.

Under normal conditions the meiotic cell divisions in *Tradescantia* occur by cell plate formation (Sax and Husted, 1936). Under the influence of dehydration cytokinesis may fail, resulting in a quadrinucleate cell. In many cases when the normal synchronization of nuclear and cell division was upset, cytokinesis occurred by a furrowing mechanism rather than by cell plate formation (fig. 8, 9).

Discussion.—The results of the present investigation indicate that dehydration inhibits normal spindle formation and consequently nuclear division. It does not, however, interfere with the division of the chromosomes themselves. Desiccation may also prevent cytokinesis even when nuclear division occurs, resulting in bi- and quadrinucleate cells. On the other hand, cell division by furrowing may occur without nuclear division. It seems clear as a result of this and numerous other experimental studies of mitosis and meiosis that chromosome division, nu-

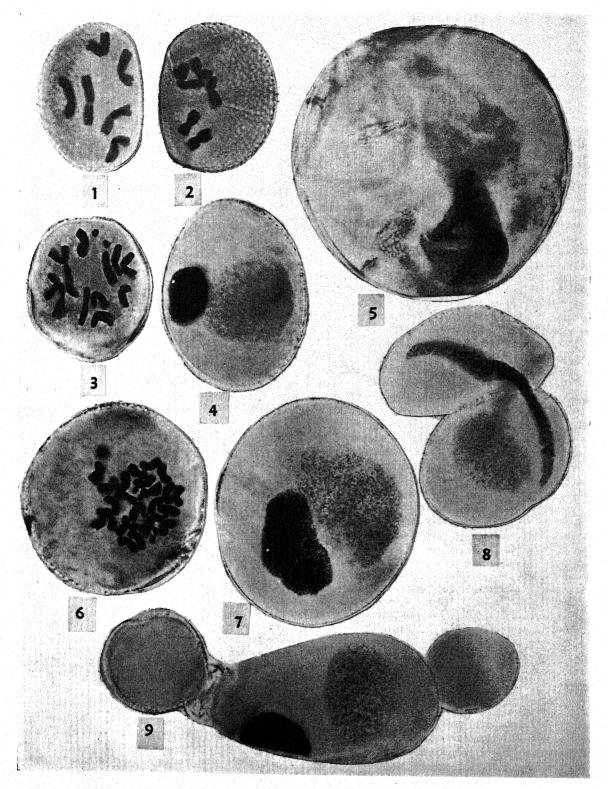


Fig. 1-9. Photographs of acetocarmine smear preparations of microspores and pollen grains of *Tradescantia* resulting from dehydration at meiosis and microspore division. All ×1200.—Fig. 1. Normal haploid microspore.—Fig. 2. Haploid microspore from bud of cut stem allowed to wilt for 24 hours. Note shortened chromosomes.—Fig. 3 and 4. Induced diploid microspore and young pollen grain, 12 and 23 days after beginning of dehydration.—Fig. 5. Induced

clear division, and cell division are three independent, though normally synchronized processes.

It is perhaps possible that some of the diploid microspores observed in these studies may have resulted not from spindle inhibition but from desynapsis followed by an equational division of the twelve univalents. A definite relation between relative hvdration and chiasma frequency at diakinesis has been demonstrated by Straub (1937) in the case of Campanula persicifolia and by Kisch (1937) for species of Oenothera. The latter author also showed the existence of a correlation between the chiasma frequency at diakinesis and the osmotic pressure of the sap of the plant parts studied. There was a definite reduction in chiasma frequency following the cessation of watering of the potted plants and a concomitant increase in the osmotic pressure of the expressed sap of the inflorescence. However, complete desynapsis was apparently not observed in any of the plants studied. It may occur, however, as a result of the application of heat (Matsuura, 1937) or colchicine (Walker, 1938) to the microspore mother cell.

The effect of cellular dehydration on mitosis and cytokinesis has been studied in some detail with living Tradescantia cells by Wada (1936a, 1936b) using stamen hairs and by Shigenaga (1937) using young petal cells. Their findings are essentially similar to those reported here for meiosis. Desiccation of the cells with dry air or plasmolysis with hypertonic sugar solutions causes the formation of didiploid nuclei and binucleate cells. The didiploid nuclei usually result from an inhibition of spindle formation and consequently of nuclear division; the binucleate cells result from the failure of wall formation.

Milovidov (1938) has made a series of observations using fixed and stained root tips of Vicia faba from germinating seedlings allowed to dry out for varying periods. This author reports the common occurrence of fusion of two or more nuclei in a multinucleate cell to produce a polyploid nucleus. However, in the case of the bi- and quadrinucleate microspores, no such fusions were found. Polyploid gametophytes at meiosis apparently result entirely from an inhibition of nuclear division, or possibly from desynapsis, rather than from a failure of cell wall formation followed by fusion of nuclei.

The reason for the failure of spindle formation or action in dehydrated cells may possibly be explained as a result of viscosity changes. Although there are some discrepancies concerning viscosity changes at various stages in the cell cycle in the animal egg as reported by Heilbrunn (1920, 1921), Chambers (1919), and Seifriz (1920), these are to a large extent reconciled by the observations of Fry and Parks (1934). The general results indicate that

cytoplasmic viscosity is normally low at the time of spindle formation during metaphase. The work on plant cells as reported by Zimmermann (1923), Kostoff (1930a), and Kato (1933) also shows a low viscosity at metaphase. Since Kostoff worked on viscosity changes during meiosis in Nicotiana L., and Kato on meiosis in Lilium L., their results are of particular significance in connection with the present investigation. Kostoff (1930b) also studied the viscosity in plant callus tissues and suggests that the multinucleate and polyploid cells often found in such growths may be the result of the increased cytoplasmic viscosity demonstrated in these cells.

Dehydration should obviously increase the viscosity of the cytoplasm of the pollen mother cells, and this increase may interfere with normal spindle formation and action, resulting in the inhibition of nuclear division.

The observations of the disturbance of normal cell divisions at meiosis seem to indicate that cytokinesis during microsporogenesis in Tradescantia may occur by two different mechanisms. As pointed out earlier, the normal mechanism is one of cell plate formation. This occurs in connection with normal spindle formation and action, a phragmoplast apparently being involved as in mitotic cell division. When, however, the spindle mechanism is disturbed by dehydration, the production of the cell plate and consequent wall formation is interfered with and often entirely inhibited. This has been very clearly demonstrated during somatic mitosis in Tradescantia by the previously cited experiments of Wada and Shigenaga. The latter worker may well be quoted on this point: "The necessity of water for the formation of the cell membrane is shown by the fact that it is inhibited by the dehydration of the connecting spindle or phragmoplast. In the dehydrated state, even when a rudimentary cell plate is visible, it disappears, and a binucleate cell results. When the medium is replaced with water at an appropriate time, the spindle or phragmoplast swells and the new membrane is formed. When the kinoplasmic body is dehydrated in a certain limited degree, the cell membrane may be formed, but not fully. These facts must show that the cell membrane formation is also closely connected with water content in the kinoplasmic body." At meiosis such failure of the spindle action and cell wall formation results in the production of dyads or monads. However, in these cases it appears that cytokinesis may now occur by a furrowing mechanism similar to that observed in many dicotyledonous genera at meiosis. Apparently the furrowing is independent of the spindle and phragmoplast, and consequently is not necessarily coordinated with nuclear division. This same effect may be caused by temperature treatment, as can

octoploid (probably) young pollen grain, 22 days after start of dehydration.—Fig. 6, 7. Induced tetraploid microspore and young pollen grain, 19 and 23 days after sealing off cut stems with paraffin. Note greatly shortened chromosomes in microspore.—Fig. 8, 9. Abnormal tetraploid pollen grains showing unequal cell division by furrowing not coordinated with nuclear division; 23 days after start of dehydration.

readily be observed in the figures accompanying the

paper by Sax (1937).

The production of normally differentiated and apparently viable diploid and tetraploid pollen grains in Tradescantia as a result of dehydration is of considerable interest in connection with the origin of polyploidy under natural conditions. Numerous agents have been successfully used in the production of polyploid plants experimentally, but to date only one-temperature variation-has been considered as having any significance in nature. It seems possible, however, that the periodically varying water supply of plants may well be an important factor in the origin of polyploid forms under natural conditions. The experiments with potted plants demonstrate that it is possible to produce polyploid pollen grains by dehydration of the entire plant. It is of course true that desiccation has a more drastic effect on the plant as a whole than temperature variation. However, it may well be that the rather sudden and extreme temperature variations which are most effective in upsetting meiosis are not of as common occurrence under natural conditions as alternating periods of drought and moisture.

### SUMMARY

The cellular water content of *Tradescantia* buds was reduced during microsporogenesis by sealing

with paraffin the ends of cut stems kept in water or by not watering potted plants for several days. Dehydration causes the suppression of the spindle at one or both of the meiotic divisions, producing diploid and tetraploid microspores and pollen grains. The larger, probably octoploid, pollen grains occasionally observed are attributed to the inhibition of the last pre-meiotic mitosis and both meiotic divisions. Cytokinesis by cell plate formation is inhibited by dehydration, in which case division by furrowing, not coordinated with nuclear division, often occurs. Nuclear division without cell division also occurs, producing bi- and quadrinucleate cells. Chromosome length in haploid microspores from buds of cut stems allowed to wilt for twenty-four hours is reduced to two-thirds that of the length in normal haploid microspores. These abnormalities are similar to those produced by many other agents such as temperature, chemicals, and radiation and result from a disturbance of the coordination of three apparently independent though normally synchronized events: chromosome-, nuclear-, and cell-division.

It is suggested that variations of the water supply of plants may be an important factor in the production of polyploid gametes and consequently of polyploid races under natural conditions.

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### INFLUENCE OF CALCIUM AND MAGNESIUM ON THE GROWTH OF CHLORELLA <sup>1</sup>

Sam F. Trelease and Millicent E. Selsam

ALTHOUGH CALCIUM is essential for the growth of all higher plants, it has long been known that Chlorella and some other green algae may be grown in culture solutions that lack this element (Molisch, 1895; Pringsheim, 1926). In this respect some of the green algae resemble bacteria and fungi, but differ from other green algae, including Spirogyra, Haematococcus, and Vaucheria, as well as from the Cyanophyceae, which, like the higher plants, require calcium for their development.

Most investigators have included calcium in the culture solution for *Chlorella* grown for physiological experiments (Warburg and Negelein, 1922, 0.0024 M; Emerson, 1929, 0.0001 M; Hopkins and Wann, 1925, 1926, 1927, usually 0.006 M; Manning, Stauffer, Duggar, and Daniels, 1938, 0.02 M). Some, however, have omitted this element (Craig and Trelease, 1937; Pratt and Trelease,

Attention is directed in the present paper to the high degree of tolerance of *Chlorella* to magnesium and to the fact that in *Chlorella* calcium does not serve as an antidote to magnesium when the latter

is present in concentrations high enough to be toxic.

Materials and methods.—Chlorella vulgaris was cultured, according to the method previously described (Craig and Trelease, 1937), in liter flasks containing 300 cc. of solution. The standard solution (similar to that of Warburg and Negelein, 1922, but with Ca omitted) contained: KNO<sub>3</sub>, 0.025 M; MgSO<sub>4</sub>, 0.020 M; KH<sub>2</sub>PO<sub>4</sub>, 0.018 M; FeSO<sub>4</sub>, 0.00001 M (with an equal concentration of potassium citrate); and stock solution of microtrophic elements, containing manganese, boron, zinc, copper, etc. (Trelease and Trelease, 1935), 14.2 cc. per liter. Experimental solutions contained, in addition, various concentrations of CaCl<sub>2</sub>, MgCl<sub>2</sub>, or MgSO<sub>4</sub>.

A mixture of 5 per cent carbon dioxide in air was bubbled continuously through the cultures, and light was supplied continuously by a water-cooled 300watt Mazda lamp about 15 cm. from the cultures. The temperature varied, but averaged about 21°C.

The cultures were seeded with approximately 35 million cells, estimated by haemacytometer counts, thus giving an initial population of about 115 cells per cmm. of culture solution. At the end of 4 days (near the middle of the grand period of growth for the control culture) the cultures were terminated,

and final populations were determined by means of a haemacytometer.

Addition of Calcium.—Excellent growth of Chlorella has been obtained in this laboratory when using a solution free from calcium (except for traces that may have been present in c.p. salts), and populations of from 7,000 to 14,000 cells per cmm. have been secured in from 3 to 5 days (Craig and Trelease, 1937; Pratt and Trelease, 1938).

The tests recorded in table 1 were made in order to determine whether the addition of CaCl<sub>2</sub>, in concentrations ranging from 0.0025 to 0.04 M, to the standard calcium-free solution would increase the growth of *Chlorella*. The data show, however, that the presence of calcium in the solution produced no significant improvement in the growth of this alga. It is evident also that *Chlorella* tolerates relatively high total salt concentrations.

Table 1. Influence of calcium chloride on the growth of Chlorella.

Culture	CaC1 <sub>2</sub>	Cell population
number	(M)	No. per cmm. % control
Control	0	12,850 100.0
1	0.0025	11,900 92.6
2	0.005	12,300 95.7
3	0.01	12,900 100.4
4	0.02	12,530 97.5
5	0.04	11,730 91.3

Tolerance of chlorella to high concentrations of magnesium.—In the tests shown in table 2, MgCl<sub>2</sub> was added to the standard solution to give concentrations of magnesium ranging from 0.02 to 0.42 molar. It may be observed that Chlo-

Table 2. Influence of magnesium chloride on the growth of Chlorella.

	.02 M MgSO <sub>4</sub> +	Cell pop	ulation
Culture number	$egin{array}{c} \mathrm{MgCl_2} \ \mathrm{(M)} \end{array}$	No. per cmm.	% control
Control	0.02	16,800	100.0
1	0.07	10,200	60.8
2	0.12	4,200	25.0
3	0.22	433	2.6
4	0.32		
5	0.42		

<sup>&</sup>lt;sup>1</sup> Received for publication February 21, 1939.

rella made significant growth (about 2.6 per cent of that of the control) in a solution containing 0.22 molar Mg (0.02 M MgSO<sub>4</sub> +0.20 M MgCl<sub>2</sub>). The data given in table 3 show that MgSO<sub>4</sub> retards growth less than MgCl<sub>2</sub>. Thus 0.42 M MgSO<sub>4</sub> reduced growth to 9.2 per cent of the control whereas 0.20 M MgCl<sub>2</sub> plus 0.02 M MgSO<sub>4</sub> reduced it to 2.5 per cent.<sup>2</sup> The difference may be due to the lower osmotic value of the MgSO<sub>4</sub>.

Table 3. Comparison of the toxicity of magnesium chloride and magnesium sulphate to Chlorella.

	Cell population				
Solution	No. per cmm.	% control			
Control	31,767	100.0			
.02 M MgSO <sub>4</sub>	810	2.5			
.20 M MgCl <sub>2</sub> ) .42 M MgSO <sub>4</sub>	2,930	9.2			

It is evident that Chlorella exhibits a remarkable tolerance to high concentrations of magnesium, even in the absence of calcium. In contrast, wheat seedlings are seriously injured by a concentration as low as 0.0025 M MgCl<sub>2</sub> if the Mg/Ca ratio exceeds 2/1 (Trelease and Trelease, 1931), and higher plants exhibit pronounced pathological symptoms when relatively low Mg concentrations are used in culture solutions that contain less than equivalent concentrations of Ca (Tottingham, 1914; Shive, 1915; Garner, McMurtrey, Bowling, and Moss, 1930).

<sup>2</sup> Other tests have shown that when Chlorella is cultured in a north window (and without additional CO<sub>2</sub>) the rate of growth in a solution containing 0.42 M MgSO<sub>4</sub> may be approximately 50 per cent of that in the control solution. Under these conditions the alga grows very slowly—the cell population after one month being only about 1,000 cells per cmm. in the control solution.

The Chlorella cells grown in 0.20 M MgCl<sub>2</sub> or 0.42 M MgSO<sub>4</sub> showed no decrease in protoplasmic contents. This is in marked contrast to the loss of all stainable cell contents observed by Bamford (1931) in roots of wheat and corn grown in solutions containing from 0.00125 to 0.02 M MgCl<sub>2</sub> and little or no Ca.

The tolerance of *Chlorella* to magnesium was used in this laboratory a number of years ago as the basis for a method of destroying protozoa in a culture that had been neglected for several months. A sufficient quantity of a strong MgSO<sub>4</sub> solution was added to give a concentration of 0.45 M. After from 12 to 24 hours, transfers made by centrifuging and decanting produced stock cultures free from protozoa.

Absence of Calcium-Magnesium antagonism.—In view of the pronounced action of calcium in counteracting the toxicity of magnesium to higher plants, one might expect that calcium would reduce the toxicity of very high concentrations of magnesium to Chlorella. Table 4, however, shows that when enough MgCl<sub>2</sub> was added to the culture solution to depress the growth of Chlorella, the addition of CaCl<sub>2</sub> had no antagonistic action in diminishing the depression of growth brought about by the MgCl<sub>2</sub>.

The results obtained with Chlorella thus resemble those of Mann (1932), who found that CaCl<sub>2</sub> did not antagonize the poisoning of Aspergillus niger by very high concentrations of MgCl<sub>2</sub>.

The colorimeter tests recorded in table 4 show that additional magnesium reduced the cell population much more than it diminished the greenness of the cultures.

General conclusion.—The higher plants (wheat, corn, tobacco, etc.), which require calcium for their development, are readily poisoned by low concentrations of magnesium in the absence of cal-

Table 4. Failure of calcium chloride to reduce the toxicity of a high concentration of magnesium salts to Chlorella.

Culture	.02 M MgSO <sub>4</sub> +	O-O)	Cell popu		
number	$egin{array}{l} \mathrm{MgCl_2} \ \mathrm{(M)} \end{array}$	$ \begin{array}{c} \operatorname{CaCl}_{2} \\ \operatorname{(M)} \end{array} $	No. per cmm.	% control	Greenness by colorimeter
Ia	0.17	0	2,360	7.4	31
2a	0.17	0.01	1,850	5.8	28
3a	0.17	0.02	1,230	3.8	15
4a	0.17	0.04	1,090	3.4	9
5a	0.17	0.08	760	2.4	3
Control	0.02	0	32,000	100.0	100
1b	0.22	0	750	3.7	
2b	0.22	0.02	300	1.5	
3b	0.22	0.04	202	1.0	
4b	0.22	0.08			
5b	0.22	0.16			
Control	0.02	0	20,350	100.0	

cium. But the lower plants (Chlorella, Aspergillus, etc.) that do not utilize calcium in their growth tolerate very high concentrations of magnesium; and in these lower plants calcium is not an antidote for magnesium, as it is in the higher plants.

### SUMMARY

The growth of Chlorella vulgaris was not increased by the addition of calcium chloride to the culture solution. In the absence of calcium this alga

tolerated high concentrations of magnesium salts, making considerable growth in a concentration as high as 0.42 molar magnesium sulphate. When enough magnesium was added to the culture solution to depress the growth of Chlorella, the addition of calcium did not diminish the toxicity of the magne-

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### THE PERENNIAL MEXICAN NAMAS 1

### C. Leo Hitchcock

Since the writer's treatment of the genus Nama in 1933 several botanists have collected interesting series of plants of the genus from the Texas-Mexican border and from the central Mexican plateau. An examination of this material has helped to clarify the status of some of the poorly known and seldom collected Mexican species.

One of the least well-understood species (so far as I am concerned, at least) is N. stenophyllum Gray. During the summer of 1938 Dr. I. M. Johnston collected and made careful field notes on several plants which are allied to that species, but which apparently are not all conspecific with it. Dr. Johnston has sent me this material together with the rest of the Mexican material at the Gray Herbarium, and careful study of these collections has con-

<sup>1</sup> Received for publication March 4, 1939.

This paper may be considered a supplement to the papers in the American Journal of Botany 20: 415-430, 518-534, 1933, in which the entire genus was reviewed, and to which frequent reference will be made in this article without recourse to citation other than pagination.

vinced me that several changes should be made in the classification which I proposed in 1933, especially as concerns the perennial species of the section Eunama.

In addition to the material from the Gray Herbarium I have borrowed collections from the United States National Herbarium (US), Texas Agricultural College, and University of California (C). My gratitude to the curators of these herbaria is hereby acknowledged.

KEY TO THE PERENNIAL SPECIES OF EUNAMA2

Corollas less than 15 mm. long.

Leaves 1.5-8 cm. long, long-petiolate; ovary 4-12-

<sup>2</sup> This key includes some plants, such as N. stenophyllum, N. canescens, and N. Havardii, which could probably be described more accurately as annuals, but which are much longer-lived than such annuals as N. demissum, N. hispidum, N. dichotomum, N. Coulteri, etc., which were treated as the annual species of Eunama.

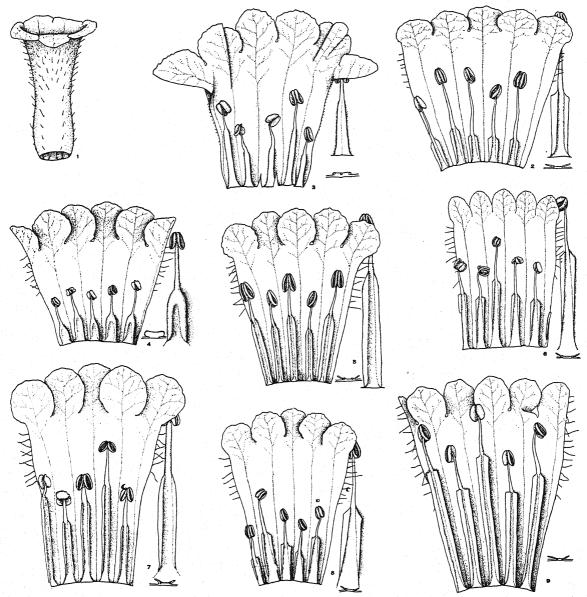


Fig. 1-9.—Fig. 1. Corolla of N. propinquum, unopened.  $\times 5$ .—Fig. 2-9. Corollas of several species of Nama opened to show attachment of stamens. All  $\times 5$ . In all except figure 9 one stamen also is shown enlarged  $\times 10$ , and in each instance a cross-section of the adnate portion of one stamen is shown at the lower right-hand corner of the corolla.—Fig. 2. N. propinquum, drawn from type.—Fig. 3. N. hispidum.—Fig. 4. N. canescens, drawn from type.—Fig. 5. N. stenophyllum, drawn from type.—Fig. 6. N. carnosum, drawn from type.—Fig. 7. N. stenophyllum, drawn from type of N. Purpusii.—Fig. 8. N. flavescens, drawn from type.—Fig. 9. N. Johnstonii, drawn from type.

Leaves seldom as much as 4 cm. long, but if that long, sessile or short-petiolate; ovary with more than 12 seeds (except in N. serpylloides and possibly in N. propinquum).

Leaves mostly opposite; plants diffusely branched, slender-stemmed, hirtellous; corollas 5-6 mm. long. Pedicels ca. 10 mm. long; plants not velvety.

N. serpylloides

Pedicels less than 5 mm. long; plants velvety.

N. serpylloides var. velutinum Leaves prevailingly alternate; plants otherwise not as in N. serpylloides.

Filaments inserted at the same level ca. ½ mm. from base of corolla, widened at point of insertion, but the adnate portion not wing-margined.

Flowers borne on long slender pedicels (or peduncles) 4-12 mm. long; free portion of filaments definitely longer than adnate portion.

Petioles broadly wing-margined, those of larger leaves at least 1 mm. broad, often decurrent on stem; pubescence villous-hirsute to hirsute.

Blades cordate or truncate at base, as broad as long; pedicels 1-4 mm. long; styles not connate; plants from woody crowns.

N. propinquum Blades acute or obtuse at base, longer than broad; pedicels mostly over 4 mm. long; styles usually somewhat connate for short distance; plants herbaceous below.

N. biflorum

Free portion of filaments definitely much longer than adnate portion; leaves mostly over 4 mm. broad (except in N. Palmeri var. argenteum).

N. Palmeri var. argenteum Free portion of filaments subequal to, or shorter than, adnate portion; leaves less than 4 mm. broad, except in N. Havardii.

Plants sericeous-villous; leaves 2-4 cm. long, 0.4-1.5 cm. broad......N. Havardii Plants with harsher pubescence than in N. Havardii (i.e., not villous); leaves 0.1-0.3 (or very rarely as much as 0.4) cm. broad.

Plants fairly shrubby at base, with irregularly furrowed bark, the larger branches 1 cm. in diameter or thicker; plants grayish-green, densely and uniformly strigose-hirsute-hispid, hairs mostly ca. 1 mm. long; stamens unequally inserted, filaments adnate % the length of the corolla (or more), free portion 1-1.5 mm. long, adnate portion with narrow winged margin.

N. Johnstonii
Plants not shrubby, or if so, not otherwise as above, either yellowish green

and sparsely strigose, or stems with dense fine pubescence in addition to sparse strigosity.

Stems sparsely strigose (the hairs nearly 1 mm. long, the bases papillosepustulose) and very densely and finely pubescent (these hairs ca. 0.2 mm. long); free portion of filaments 1-2 mm. long, the adnate portion 2-4 mm. long, but the free-margined edges not extending all the way to the base Stems uniformly pubescent, or at least without fine pubescence as short as 0.2 mm. in length; filaments (except in N. flavescens) not as in N. carnosum, the free-margined edges of adnate portion usually extending practically to base of corolla.

Plants slightly shrubby at base, with roughened bark on main branches, densely granular-glandular all over; leaves yellowish-green, sparsely strigose; sepals 4-6 mm. long; corollas exserted 3-4 mm.

### EMENDED TREATMENT OF SPECIES AND VARIETIES

N. serpylloides var. velutinum C. L. Hitchc. var. nov.—Habit of N. serpylloides, but entire plant densely hirtellous-velutinous, hence grayish-green; branches thicker than in the species; leaves all opposite, obovate or obovate-oblanceolate, 5-9 mm. long, 2-4 mm. broad, thick and fleshy, revolute; flowers sessile or on pedicels 1-4 mm. long; sepals linear-oblanceolate, 2-3 mm. long, rounded; corollas 4-6 mm. long, the tube yellowish, lobes pinkish; filaments subequally inserted ca. 1 mm. above base of corolla, the bases widened slightly and very narrowly wing-margined at point of insertion, the wings not extending to the base of the corolla, the free filaments 1-1.5 mm. long; styles ca. 1.5 mm. long; capsules 10-20-seeded; seeds as in the species. (Planta habitu N. serpylloidei similis sed velutina; pedicellis 1-4 mm. longis; foliis crassis, 5-9 mm. longis, 2-4 mm. latis.)

Type; one mile south of Hermanas, on road from Piedras Negras south to Monclova, Coahuila, Mexico, on dry heavy alkaline soil on terrace near saltmarsh, Aug. 22-24, 1938, I. M. Johnston 7063 (Gray Herbarium). Other material seen; 2 miles west of Cuatro Cienegas, road from Monclova westward to beyond Cuatro Cienegas, Coahuila, Mexico, Aug. 25, 1938, Johnston 7126 (G).

That the plants represented in these two collections are closely related to N. serpylloides, there can be no doubt, the species as a whole being the only Nama characterized by opposite leaves; however, although Johnston noted that his plants were collected on very alkaline soil, I believe that the differences between them and the species, proper, are not entirely due to ecological conditions.

N. propinguum Morton and Hitchcock spec. nov.—A gravish, densely hirsute perennial nearly a foot high, from a definitely woody crown; leaves silvery-gray, cordate-deltoid, the larger blades about 2 cm. long and as broad, the veins very prominent, the petioles about 1 cm. long, narrowly wing-margined, the margins sometimes somewhat decurrent on the stems; flowers borne singly or in pairs on slender peduncles and pedicels 5-10 mm. long, the pedicels alone usually 1-4 mm. long; sepals linear, ca. 5 mm. long; corollas narrowly tubular-obconic, 8-9 mm. long, the throat pubescent on the exterior; stamens unequal, slightly over half as long as corolla-tube, filaments unequally inserted 1-2 mm. from base of corolla, the adnate portion flared just below upper limit of adnation, then narrowed to base, the margins free; styles 3-4 mm. long, not connate at base; ovaries with more than 20 ovules, but mature seeds not seen.

Planta perennans, suffrutescens, 15-25 cm. alta, cinereo-hirsuta; foliis cordato-triangularibus, usque ad 2 cm. longis et 2 cm. latis, venis prominentibus; petiolis ca. 1 cm. longis, alatis, subdecurrentibus; floribus in cincinnis axillaribus, 1-2-floris; pedicellis 1-4 mm. longis; sepalis ca. 5 mm. longis, linearibus; corollis 8-9 mm. longis; staminibus per 1-2 mm. cum corolla coalitis, alato-dilatatis; stylis non connatis; ovulis numerosis, seminibus mihi ignotis.

Type; Rancho Agua Dulce, dry arroyo on the slopes of the Sierra de San Manuel, Musquiz, Coahuila, Mexico, June 28, 1936, Wynd & Mueller 333 (U. S. Nat. Herb. No. 1639772). Isotype at Gray Herbarium.

The nearest relative of this species is undoubtedly *N. biflorum* Gray, a species which differs from *N. propinguum* as follows:

N. biflorum

veins of leaves only grayish; pubescence villoushirsute;

leaves ovate to spatulate; petioles broadly winged, always decurrent on stems;

pedicels usually over 4 mm. long; styles usually slightly connate;

annual or biennial, crown not woody.

N. propinguum

whole plant silvery-gray; pubescence hirsute, much harsher than in biflorum; leaves cordate-deltoid; petioles not so broadly winged as in biflorum, scarcely decurrent on stems;

pedicels usually less than 4 mm. long; styles not connate;

perennial, the crown quite woody.

Mr. C. V. Morton, of the National Herbarium, tentatively identified this Nama as a new species, and it is with his permission that I use his herba-

rium name and publish the species under our joint authorship.

N. xylopodum (Woot. & Standl.) C. L. Hitche. Amer. Jour. Bot. 20: 428. 1933.—My description of this species should be emended to note that the leaves are sometimes as much as 15 mm. long and 7 mm. broad.

N. spathulatum T. S. Brandegee, Zoë 5: 253. 1908.—Upon reexamination of the material of N. spathulatum and N. Palmeri, I am more inclined than ever to believe that those two species are not specifically distinct. However, the differences I pointed out (p. 425) still stand in the light of present collections, although the types of the two species—namely, Purpus 2584, from Cerro de Paxtla, Puebla, and Palmer 856, 25 miles southwest of Monclova, Coahuila—are more than superficially alike.

N. Johnstonii C. L. Hitchc. spec. nov.—A gravish-green perennial from a woody base, the plants rounded shrubs 20-35 cm. tall, 15-30 cm. in diameter; basal branches as much as 1.5 cm. thick, decidedly woody, with thick furrowed bark, devoid of leaves for 5-15 cm.; whole plant somewhat mealyglandular and strigose-hirsute-hispid, the hairs of older leaves with papilliform bases, mostly ca. 1 mm. long; leaves linear, 10-25 mm. long, 1-2 mm. broad, revolute; flowers 1-several per peduncle, mostly at ends of short herbaceous branches on pedicels 1-5 mm. long; sepals linear, ca. 1 cm. long, 0.5-0.75 mm. broad; "corollas white, 3 purple lines on each lobe," 10-12 mm. long, hirsutulous on the exterior; filaments unequally inserted, adnate for ca. 2/3 length of corolla-tube (or for greater distance), free portion 1-1.5 mm. long, adnate portion widened and with narrow winged margins for entire length; styles distinct to base, 4-5 mm. long; seeds numerous (50-100?), light brown, ca. 0.3 mm. long, very irregularly many-sided, finely alveolate, the edges somewhat granular.

Planta suffrutescens, perennans, 20–35 cm. alta, 15–30 cm. lata; caulibus usque ad 1.5 cm. crassis, lignescentibus; plantis glandularibus, strigosis, hirsuto-hispidis; foliis linearibus, 10–25 mm. longis, 1–2 mm. latis, revolutis; cincinnis 1–4-floris; pedicellis 1–5 cm. longis; sepalis linearibus, ca. 10 mm. longis, 0.5–0.75 mm. latis; corollis 10–12 mm. longis; staminibus per 3–5 mm. cum corolla coalitis, partibus adnatis alato-dilatatis; stylis 4–5 mm. longis, non connatis; seminibus 50–100, brunneis, ca. 0.3 mm. longis, minute alveolatis et granularibus.

Type: 6 miles west of Viesca, Coahuila, Mexico, on limestone cliffs in steep canyon, Sept. 17, 1938, I. M. Johnston 7740 (Gray Herbarium). Other material seen: State of Chihuahua (probably from the great canyon near Chihuahua City, according to I. M. Johnston), Pringle 120 (G, US).

This species, as represented by the Pringle collection, was referred to D. stenophyllum by me and by Macbride. However, Dr. Johnston pointed out the similarity of his collection to that of Pringle and called attention to the shrubby nature of the plant and to the fact that it is found on limestone

cliffs, whereas N. stenophyllum is found chiefly on

gypsum soil.

Since there is good reason for considering this shrubby plant to be a distinct species (for comparison with N. stenophyllum see under the latter species), I have restudied the other forms which I included under N. stenophyllum in my former paper, and now believe that it would be more consistent to recognize N. carnosum and N. flavescens as distinct also, the four species (N. carnosum, flavescens, stenophyllum, and Johnstonii) undoubtedly being of common origin and rather closely related.

N. carnosum (Woot.) C. L. Hitchc. comb. nov.— Conanthus carnosus Woot., Bull. Torr. Bot. Club 25: 262. 1898. Andropus carnosus (Woot.) Brand, Fedde Rep. Spec. Nov. 10: 281. 1911, and Pflanzenr. 4<sup>251</sup>: 163, fig. 32. 1913. N. stenophyllum var. egenum Macbride, Contr. Gray Herb. 49: 44. 1917; Hitchc., l.c. 425.

Unfortunately the only two collections of this plant that seemingly are extant (namely Havard 15, from bluffs of Delaware Creek east of Guadalupe Mts., Texas, the type of Macbride's variety egenum, and Wooton 164, from White Sands, Dona Ana Co., New Mexico, type collection of N. carnosum) are both past anthesis; therefore all observations concerning the flowers have perforce been made from a few fragmentary undersized blossoms. As I pointed out previously, the filament bases are not bifid, although they seem to be because of the fact that the free-margined adnate filament bases do not extend to the base of the corolla. My previous illustration (l.c. plate 27, fig. 17) for N. stenophyllum was made from Wooton 164. After studying the 1938 collections made by Johnston, it is clear that the above-mentioned figure, although approximately correct for N. carnosum, does not accurately represent the stamen structure in N. stenophyllum, proper, but that the figure for N. Purpusii (fig. 32) more nearly represents their true nature. I have accordingly redrawn the corollas of N. stenophyllum and its allies for purposes of comparison.

Since N. stenophyllum is apparently never woody at base, whereas N. carnosum is woody, I believe that the latter can justifiably be maintained as a species characterized by its woody crown, long branches that are simple below, and by the peculiar pubescence described in the key. In all these respects it more closely resembles N. flavescens (N. stenophyllum var. flavescens) than it does N. stenophyllum itself.

It is to be hoped that collectors will make a special attempt to collect this species, as it surely must have been seen in the field by other botanists, but might easily be passed up as unsatisfactory for collecting due to its dark color and "dried-up" appearance.

N. flavescens T. S. Brandegee, Zoë 5: 254. 1908, Brand, l.c. 158. N. stenophyllum var. flavescens (Brandegee) C. L. Hitchc. l.c. 425.—Since the stamen bases of those plants are seemingly more like

those of *N. carnosum* than like those of *N. stenophyllum*, and because the plants are apparently somewhat woody at base, I believe that they, too, can reasonably be maintained as a species, rather than as a variety of *stenophyllum*, although additional material might show that such is not the case.

N. stenophyllum Gray ex Hemsl. Biol. Cent.-Am. Bot. 2: 361. 1882; Brand, l.c. 145; Hitchcock, l.c. 425. Conanthus stenophyllus (Gray) Heller, Cat. N. Am. Pl. 6. 1898. Marilaunidium stenophyllum (Gray) Kuntze, Rev. Gen. Pl. 2: 434. 1891. N. Purpusii T. S. Brandegee, Univ. Cal. Pub. Bot. 4: 186. 1911; Brand, l.c. 159; Hitchcock, l.c. 426.

After comparing the types of N. Purpusii and N. stenophyllum with the material collected last summer by Johnston, I can see that I misinterpreted the nature of the filaments of the latter and that they are the same as those of N. Purpusii. Therefore the only real difference which I supposed existed between these two "species" does not really exist at all. Table 1 shows why I consider these two to be conspecific and also shows the difference between N. stenophyllum and its closest allies.

N. canescens C. L. Hitchc. spec. nov.—A decumbent to ascending rounded annual or biennial 5-20 cm. tall, grayish, appressed-hispid throughout, the hairs mostly ca. 1 mm. long, some slightly shorter and more slender, pubescence also present on stems; stems several from base, freely branched, very leafy; leaves linear, 10-25 mm. long, 1-1.25 mm. broad (nearly terete?); flowers mostly in terminal and axillary few-flowered bracteate cymes; pedicels 1-2 mm. long; sepals free to base, linear, 4-6 mm. long at anthesis, to 10 mm. long in fruit; corolla pink, ca. 8 mm. long; stamens subequally inserted 1-2 mm. from base of corolla, the longer ones ca. ½ as long as corolla, free portion of filaments ca. equal to adnate bases or slightly longer, the adnate portions broadened to nearly 3 times the width of the free filament, the edges thickened and scarcely free; styles not connate; seeds brown, irregularly many-sided, alveolate and somewhat granular, ca. 0.4 mm. long.

Planta habitu N. stenophyllo similis sed hispida; foliis linearibus, 10-25 mm. longis, 1-1.25 mm. latis; corollis ca. 8 mm. longis; staminibus per 1-2 mm. cum corolla coalitis, partibus liberis 1-2 mm. longis, partibus adnatis dilatatis sed marginibus non alatis; seminibus brunneis, ca. 0.4 mm. longis.

Type: 38 miles south of Matehuala, San Luis Potosi, Mexico, on disturbed soil of gypsum plain, Sept. 10-11, 1938, I. M. Johnston 7510 (Gray Herbarium). Other material seen: 2 miles south of Cedral, San Luis Potosi, on gypsum plain, Sept. 11-12, 1938, Johnston 7584 (G).

This is a rather puzzling species, resembling N. hispidum in some respects and N. stenophyllum in others, but it is probable that it is much more closely related to the latter than to the former, as shown in table 2 below.

TABLE 1.

stenophyllum	Purpusii	Johnstonii	flavescens	carnosum
Sturdy annuals or biennials, bases not woody; stems very leafy, fascicles and reduced branches in most axils.	Same.	Woody-based perennial; branches as much as $1\frac{1}{2}$ cm. thick, with grayish bark, irregularly branched but without the numerous short branches of stenophyllum.	Shrubby-based perennial, bark thick, branches many, simple, without fascicles and reduced branches in the axils of the larger leaves.	Base woody, rough barked, several long branches from base, these branched only near tips
Plant grayish, hispid- strigose-hirsute throughout, longer hairs 1-1.5 mm. long, often with shorter hairs also present, all hairs straight and fairly stiff, not papillose or glandular.	Same.	Grayish-green, strigose hirsute-hispid, hairs on older leaves with papil- liform bases; hairs mostly ca. 1 mm. long; plant somewhat mealy- glandular throughout.	Yellow-green, very densely granular-glan- dular all over, sparsely strigose, the hairs less than 1 mm. long, some- what papilliform.	Sparsely strigose, th hairs ca. 0.75 mm. long papilliform-pustulose stems densely pubescen also, with short hairs no over 0.25 mm. long.
Leaves revolute, linear, 10-50 cm. long, 1-3 mm. broad.	Same.	Revolute, linear, 10-25 mm. long, 1-2 mm. broad.		Linear, revolute, 10-3 mm. long, 1-1.5 mm broad.
Pedicels 1-3 mm. long.	Same.	1-5 mm. long.	1-3 mm. long.	1-2 mm. long.
Sepals 7-9 mm. long, linear-spatulate.	8–10 mm. long.	Linear, ca. 10 mm. long.	Linear, 4-6 mm. long.	Linear, ca. 7 mm. long.
Corolla 7.5-9 mm. long, not exserted.	8–10	10-12 mm. long, often exserted.	8-9 mm. long, exserted.	Ca. 7 mm. long?
Stamens unequally inserted, adnate portion 2-5 mm. long, free portion 1/2 as long to as long as adnate, free-margined portion.	Same.	Much same as steno-phyllum but adnate nearly % length of corolla.	Unequally inserted, adnate portion 1-3 mm. long, free portion ca. equal to adnate portion, adnate portion, adnate portion expanded, with narrow free margins running not quite to base of corolla.	Much same as in flaves cens.
Seeds brownish, 0.3-0.5 mm. long, very irregularly compressed, finely alveolate, the edges somewhat granular.	Same.	Same.	Same.	Same.

TABLE 2.

N. stenophyllum	N. canescens	N. hispidum
Vigorous long-lived annual or biennial.	Same as stenophyllum.	Short-lived annual.
Leaves linear, 1-1.25 mm. broad.	Same.	Variable, but mostly over 1.5 mm. broad.
Strigose-hispid-hirsute.	Hispid.	Hispid to hirsute.
Sepals lanceolate, 7-10 mm. long.	Linear, 4-10 mm. long.	Linear or linear-lanceolate, 4-7 mm. long.
Corollas 8-10 mm. long.	Ca. 8 mm. long.	8-15 mm. long.
nate 2-5 mm.; free filaments 1/3 as	adnate for 1-2 mm.; free filaments equal to or longer than adnate por-	Ca. ½ as long as corolla, adnate 1-4 mm., free portion longer than adnate portion, the adnate bases neither winged nor free-margined.
Seeds brown, many-sided and flat- tened, somewhat granular.	Same.	Yellow, ovoid, smooth but alveolate- reticulate.

### NOTES ON THE ANNUAL SPECIES

Nama hispidum Gray, Proc. Am. Acad. 5: 339. 1861.—Two of Dr. Johnston's collections—namely, Johnston 7406, Hacienda de Sierra Hermosa, Zacatecas, and Johnston 7633, from 6 miles north of La Ventura, Coahuila—are unusual in that the plants are prostrate and very narrow-leaved. However, the pubescence, flowers, and seeds are those of N. hispidum, and I hesitate to assign them any distinctive status, nomenclaturally, in such a perplexing species. Both series of plants were growing on gypsum soil.

Nama Coulteri var. Pringlei (Rob. & Greenm.) C. L. Hitche. l.c. 526.—A further difference between this plant and N. Coulteri is that the stems and calyces are densely gray hirsute, the hairs being 1 mm. long or longer. I believe that it would probably be wisest to accord the entity specific status as

was done by Robinson and Greenman, Proc. Am. Acad. 32: 38, 1896.

### SUMMARY

The perennial Mexican species of Eunama have been restudied and based on recent collections of Mexican material; 3 new species, N. Johnstonii, N. canescens, and N. propinquum, and one new variety, N. serpylloides var. velutinum, are described.

It is now proposed to maintain N. carnosum and N. flavescens as valid species; N. Purpusii is reduced to synonymy under N. stenophyllum; and it is suggested that N. Pringlei might more consistently be treated as a species than as a variety of N. Coulteri.

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### RELATION OF STOMATAL OPENING TO WATER SOAKING OF TOBACCO LEAVES <sup>1</sup>

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SINCE THE theory has been advanced that water soaking of leaves is a necessary prerequisite to the development of "epidemic" wildfire and late-season blackfire of tobacco (Clayton, 1936, 1937), further information was sought concerning the factors which modify the susceptibility of leaves to water soaking. Clayton (1936) reported that leaf maturity, height of topping, presence of leaf injuries, fertilization, and surface of leaf exposed modified the susceptibility of tobacco leaves to water soaking, but did not offer any explanation as to how these factors affected susceptibility. In the process of soaking a leaf with a strong spray of water, the water presumably enters the intercellular spaces by way of stomata or leaf injuries. The suggestion thus occurred that the condition of the stomata might have some bearing on the susceptibility of leaves to water soaking.

In the present studies it was found that leaves water soaked very slowly (1) when light intensity was low late in the afternoon, (2) when leaves were shaded, (3) when leaves were wilted or beginning to wilt, or at any other times when the stomata might be expected to be closing or closed. It is the purpose of this paper to report studies which indicate that the susceptibility of tobacco leaves to water soaking is determined largely by the degree of opening and condition of the stomata.

Methods.—Leaves were water soaked by directing a stream of water from a glass tube against the

<sup>1</sup> Received for publication February 10, 1939. The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director. lower surface. All tests were made on the lower surface, because storm injury in the field is more prevalent on leaves whose lower surfaces are exposed after the leaves are turned over by wind. To produce the stream of water, a simple apparatus was used: a glass capillary tube 2 cm. long with a bore of .75 mm. was connected by means of a heavy walled rubber tube to a ½-inch iron pipe fitted with a pressure gage and valve; the pipe was connected to the water line by a garden hose. Water pressure could be kept fairly uniform by means of the gauge and valve. Susceptible leaves could be water soaked easily without mechanical injury when the nozzle was held 30 cm. from the leaf surface with a pressure of  $7\frac{1}{2}$  lbs. per square inch.

The method used to determine the susceptibility of a leaf to water soaking was as follows: a flat board was placed against the upper surface of the leaf; a flat piece of sheet metal with a hole 4 cm. square in the center of it was then placed over the lower surface of the leaf; a stream of water was directed at the leaf through this opening with the nozzle 30 cm. away; the stream of water was moved across the exposed leaf from side to side as rapidly as the tissues became soaked until the whole area was soaked. The time required to water soak the 16 cm.2 area was recorded to the nearest 5 seconds, if less than a minute was required, or to the nearest quarter minute at the longer periods. Because a difference was observed in the susceptibility of base and tip of some leaves, three areas of each leaf were water soaked: base, middle, and tip.

The alcohol method of Lloyd (1908) was used in examining the stomata. To facilitate comparison of

the condition of the stomata at different times, an index of the degree of stomatal opening was used; this index, expressed as K, is the ratio of the average actual width to the average potential length of the opening of 20 measured stomata.

These studies were confined to White Burley tobacco grown in the greenhouse in half-gallon pots or in the ground bench. A coat of whitewash on the glass of the house reduced the intensity of sunlight.

Effect of time of day and intensity of sunlight on susceptibility to water soaking.—Repeated observations and tests showed that leaves water soaked much more slowly late in the afternoon than in the forenoon or early afternoon. At 9 a.m., April 13, 1938, the time required to water soak 16 cm.<sup>2</sup> of each of eleven leaves on a plant in the ground bench was determined. Other 16 cm.<sup>2</sup> areas of the same leaves were water soaked at 4 p.m. the same day and at 9 a.m. the next day. On both mornings the leaves water soaked in about 1/6 the time required late in the afternoon. The middle leaves water soaked most readily, and the tip leaves least readily. This was the case throughout these studies.

Hourly records were then made on the degree of opening of stomata and the time required to water soak 16 cm.<sup>2</sup> over a 24-hour period beginning at 8 a.m., May 5, 1938. The test was made on middle leaves of mature plants in bloom in the ground bench, and on middle leaves of small potted plants

with 7 to 10 leaves per plant. The soil was kept moist throughout the test so that there was no wilting during the hot part of the afternoon.

From 8 a.m. until 4 p.m. the leaves water soaked readily, and most of the stomata were open. At 5 p.m. the leaves water soaked more slowly, and the stomata were beginning to close. During the night the leaves water soaked very slowly, and most of the stomata were closed or nearly so. At 6 a.m. some of the stomata were opening, and the leaves water soaked more readily. At 7 a.m. most of the stomata were wide open, and the leaves water soaked rapidly. This experiment was repeated on September 10, 1938. The hourly observations presented in table 1 show that leaves water soaked easily during the hours of daylight when most of the stomata were open. At night when most of the stomata were nearly closed, leaves water soaked much more slowly.

Since the coming of nightfall and darkness tended to induce closure of stomata and an accompanying increased resistance to water soaking, it was thought that artificial darkness might have a similar effect. Therefore, on August 2, 1938, at 9:45 a.m., the susceptibility to water soaking and the degree of stomatal opening was determined for a leaf of a potted plant (15 leaves). The plant was then put into a dark room. At 15-minute intervals for an hour, 20 stomata were measured and the time required to water soak 16 cm. 2 determined. At the end

Table 1. Effect of time of day on susceptibility of tobacco leaves to water soaking, and on the degree of opening of stomata.

	Light in foot	Percentage relative		Time in sec. required to water soak 16 cm. <sup>2</sup>		Stomatal width in		
Time	candles	Temp. °C.		Base	Middle	Tip	microns	K
8 a.m.	300	20,0	90	20	15	15	4.5	.24
9	1000	22.2	80	15	15	15	4.5	.25
10	2000	27.2	72	15	15	15	4.5	.23
11	2500	31.1	67	10	10	10	6.4	.31
12	3000	31.1	65	10	10	10	5.7	.30
1 p.m.	3000	32.2	65	10	10	10	5.4	.33
2	2500	31.1	65	10	10	10	6.7	.31
3	2500	31.1	65	15	15	15	5.4	.27
4	1800	30.0	65	15	15	10	4.4	.30
5	400	28.9	67	20	20	20	3.7	.21
6	10	27.7	79	60	60	60	2.3	.12
7	0	26.1	78		300		1.0	.05
8	0	24.4	79		300		1.2	.06
9	0	23.9	81		300		.6	.03
10	0	22.8	84		300		1.7	.09
11	0	22.8	84	4.5	300		2.4	.11
12	0	22.2	87		300		1.1	.06
1 a.m.	0	22.2	87		300		1.3	.07
2	0	22.2	87		240		1.8	.08
3	0	22.2	87		240		2.9	.18
4	0	22.2	87		120		2.2	.10
5	2	22.2	87		240		1.7	.09
6	400	22.8	85	25	20	20	5.4	.32
7	2000	27.2	79	15	10	10	5.7	.31
8	2500	27.8	70	15	10	10	4.1	.29

of the hour the plant was returned to the green-house. For another hour stomata were measured and the time required to water soak 16 cm.<sup>2</sup> determined at 15-minute intervals. Table 2 shows that the stomata were closed 15 minutes after being placed in the dark and remained closed until the plant was brought into the greenhouse, when they began to open. The susceptibility to water soaking was closely correlated with the degree of stomatal opening. This experiment was repeated several times with similar results.

Table 2. Relation of light and artificial darkness to opening and closing of stomata and susceptibility to water soaking.

Sunlight in Time foot candles				
a.m.				
9:45	1800 (cloudy)	15	.29	
10:00a	0	More than 360	.06	
10:15	0	More than 360	.06	
10:30	0	More than 360	.08	
10:45	0	More than 360	.04	
11:00 <sup>b</sup>	6000 (bright)	45	.21	
11:15	5000	20	.26	
11:30	5500	15	.36	
11:45	5000	15	.35	

<sup>a</sup> Plant removed to dark room at 9:46.

b Plant removed from dark room at 10:46.

Shaded parts of leaves did not water soak as readily as those parts exposed to direct sunlight, as is shown in the following experiment. A leaf of a plant in the ground bench was partly shielded from light by a paper mask for one hour. The mask was then removed and the entire leaf sprayed from the

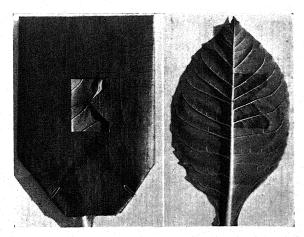


Fig. 1. Effect of shading on susceptibility to water soaking. Left. Paper mask exposing only a part of leaf to sunlight. Right. Leaf masked one hour then sprayed with stream of water. Only the part previously exposed to light became water soaked.

lower side. Only the part exposed to light became water soaked (fig. 1). The stomata on the exposed part of the leaf were open, K being .34, while those on the masked part were nearly closed, K being .06. This experiment was repeated several times with the same result.

EFFECT OF LOW LIGHT INTENSITY DURING THE DAY ON WATER SOAKING AND STOMATAL OPENING. On August 11, 1938, hourly observations were made on the degree of opening of stomata and the ease of water soaking of leaves. The morning was partly cloudy; stomata were open and leaves water soaked easily. About noon it began to rain and remained cloudy and dark all afternoon. At 1 p.m. stomata were partly closed, and leaves water soaked about nine times more slowly than in the morning. The stomata were partly closed all afternoon, and leaves water soaked slowly. With nightfall stomata did not close as they had been observed to do on previous nights but remained partly open all night. Leaves water soaked more rapidly than on previous nights when stomata had been nearly closed (table 3). After midnight a full moon was shining. Whether this had any effect on keeping stomata open is not known.

Loftfield (1921) observed that in alfalfa a night opening of stomata occurred when some factor such as water shortage caused stomata to close in the afternoon. Presumably tobacco leaves behave in a similar manner. A partial closure of stomata in the afternoon caused by low light intensity seemed to induce a partial opening throughout the night.

RESISTANCE OF WILTING LEAVES TO WATER SOAK-ING.—Leaves beginning to wilt usually could not be water soaked. On June 20, 1938, a plant in the ground bench began to wilt at 11 a.m. Several leaves were drooping at the tip and margins. When the lower surface of one of these leaves was sprayed in the usual manner, the central portion of the leaf water soaked rapidly, but the tip and margins only very slowly. The stomata in the center of the leaf were open, K being .39; those on the margin of the leaf were nearly closed, K being .10. However, sometimes even wilting leaves water soaked rapidly over the entire surface. At such times the stomata were found to be uniformly open.

RELATION OF STOMATAL OPENING TO WATER SOAK-ING IN OLD LEAVES AND IN YOUNG TIP LEAVES.—Clayton (1936) reported that basal leaves water soaked more readily than young tip leaves on the same plant. The writers found that the middle leaves of Burley tobacco water soaked more rapidly than the low, more mature leaves (table 4). In view of the fact that the degree of stomatal opening was seen to be a factor in determining the ease of water soaking and that Loftfield (1921) had reported stomata in old yellow leaves of alfalfa to be permanently closed, the stomata in old, basal, yellowing leaves were examined. It was found that the majority of the stomata in such leaves were always closed or very nearly so. Furthermore, some of them

Table 3. Effect of sunlight and a cloudy afternoon on susceptibility of tobacco leaves to water soaking, and on degree of stomatal opening.

	Light		Percentage	Time in sec. required to water soak 16 cm. <sup>2</sup>			Stomatal width in	
Time	in foot candles	Temp. °C.	relative humidity	Base	Middle	Tip	microns	К
8 a.m.	1500	29.6	80	10	10	10	5.4	.25
9	1500	30.0	85	10	10	10	5.8	.33
10	2800	31.5	85	10	10	10	5.6	.31
11	1800	31.5	70	10	10	10	5.7	.32
12	1400	31.8	75	10	10	10	5.2	.30
1 p.m.	85	26.5	85	105	90	90	2.6	.12
2	90	26.0	95	45	45	45	4.8	.24
3	60	25.8	96	75	75	60	4.2	.21
4	50	25.2	96	105	105	105	2.8	.14
5	45	25.5	96	180	150	135	2.9	.13
6	25	25.5	99	150	150	150	3.1	.14
7	1	25.5	99	150	150	150	2.1	.10
8	0	25.0	98	120	120	120	3.7	.16
9	0	25.0	99	120	120	120	2.2	.10
10	0	25.0	99	75	75	90	2.7	.12
11	0	25.0	99	90	60	60	4.5	.21
12	0	25.0	99	90	90	90	3.9	.21
1 a.m.	0	25.0	98	75	60	75	4.6	.23
2	0	24.5	97	90	90	90	2.8	.14
3	0	23.0	96	60	90	60	4.3	.20
4	0	22.0	96	60	60	55	3.9	.20
5	40	22.0	96	55	60	60	4.7	.21
6	400	22.6	96	60	60	55	4.2	.19
7	1100	25.8	90	15	15	10	5.2	.26
8	1600	25.8	65	10	10	10	4.5	.23

Table 4. Susceptibility to water soaking of basal, middle, and tip leaves of 4 representative plants.

	Time in seconds required to water-soak 16 cm.2					
Leaf no. (Numbered from bottom)	Plant 1 April 12/38	Plant 2 April 13/38	Plant 3 April 14/38	Plant 4 April 15/38		
1	75	240	120	180		
2	105	150	75	120		
3	105	105	60	90		
4	105	75	25	35		
5	120	90	60	50		
6	30	75	45	50		
7	25	90	25	50		
8	50	90	45	50		
9	20	90	20	30		
10	25	50	45	40		
11	20	30	25	15		
12	20	30	20	15		
13	15	20	20	20		
14	25	20	20	20		
15	20	25	15	15		
16	25	15	20	15		
17	25	20	20	15		
18	20	20	50	10		
19	30	20	40	15		
20	50	30	240	25		
21	120	105	240	60		
92	240	240		240		
23	240			240		

appeared to be non-functional, with degenerating guard cells (fig. 2).

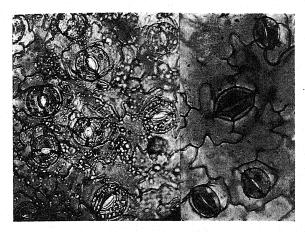


Fig. 2. Left. Normal stomata from middle leaf of a Burley plant. Right. Stomata from basal yellowing leaf of a Burley plant. Most of the stomata are nearly closed; some of the guard cells are degenerating. Both taken from leaves at 11 a.m. of a sunny day.

The ripening leaves of a low-topped plant have small, yellow patches on a green background. These yellow areas water soaked slowly even when the green portions of the leaf water soaked rapidly. In the green regions the stomata were large and still functional, most of them being open during the day. The stomata in the yellow areas, however, were closed, for the most part, and many of them were structually deformed, resembling those on old, yellow, basal leaves of untopped plants.

Young tip leaves consistently water soaked much more slowly than more mature middle leaves (table 4). This is in accord with Clayton's results. Microscopic examination showed stomata on tip leaves to be less fully developed than those on middle leaves. This is in agreement with the observation of Clinton and McCormick (1922). These workers reported that they were able to produce abundant wildfire infections on uninjured tissues of mature or nearly mature leaves inoculated by spraying, when environmental conditions were favorable for the opening of stomata. However, when immature leaves were sprayed, infections rarely occurred. This "indicates that the entrance takes place through the stomates which in these leaves are not so fully developed or liable to be open." Furthermore, the tips

of such young leaves could be water soaked easily when the bases of the same leaves could not be. Since the tip of a leaf is believed to become mature before the base (Avery, 1933), it was felt that the stomata might be more fully developed at the tip. Examination of stomata at the tip and base of young leaves shows this to be the case.

Limited observation on several other species of plants indicates that some other herbaceous plants can be water soaked by spraying with a strong stream of water and that leaves in the sun water soak more rapidly than those in the shade.

### SUMMARY

Susceptibility of tobacco leaves to water soaking by a stream of water was determined largely by the degree of opening and condition of stomata. During the day stomata were open, and leaves could be water soaked easily and rapidly. At night, stomata usually were closed or nearly closed, and leaves water soaked only with difficulty. Wilting leaves and leaves shaded naturally or artificially water soaked slowly, and stomata were closed or nearly so.

Middle leaves of mature Burley plants water soaked more rapidly than tip leaves or basal leaves. Stomata were not fully developed on tip leaves. On basal leaves stomata were always closed or nearly so, some being deformed with degenerating guard cells.

The method described in this paper or some modification of it may be used as an indirect method for studying stomatal behavior.

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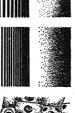
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### DRAWING GUIDE FOR ZINC ETCHINGS

Letters, lines, and dots in relation to reduction for the printed page. Top-Reduction to 1/4. Middle-Reduction to 1/2. Bottom-Original size.

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duction, but that small black dots and small white spaces between black lines or dots may be lost. If placed too close together, dots and lines produce black ing rather open. The degree of reduction needs to be Note that thin black lines hold up fairly well in reblotches when the drawing is reduced. Keep the shadknown before the drawing is inked in,

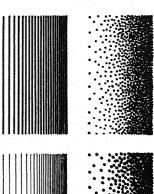
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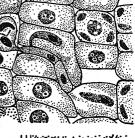
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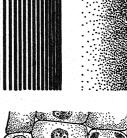
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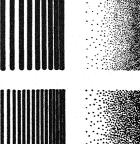
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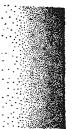












# VENTRAL SURFACE SPECIALIZATIONS OF CONOCEPHALUM CONICUM 1

# Marjorie McConaha

THE THALLOID liverworts with limited tissue specialization are peculiarly dependent upon external conduction of water. A study has been made of Conocephalum conicum (L.) Dumort. with particular attention to its ventral differentiations in their relation to capillarity. This paper presents an analysis of these organs with respect to their number, position, and surface area, as well as consideration of their interrelations.

OBSERVATIONS.—The thallus of Conocephalum conicum is differentiated into a conspicuously thickened midrib and thin lateral wings. The midrib ranges from 1.0 to 1.4 mm. wide or one-eighth to one-tenth of the thallus width. It is about 0.35 mm. in vertical thickness in contrast to the thallus wings which taper from about 0.2 mm. near the midrib to a single row of cells at the margin of the thallus. Except for a few scattered rhizoids on the wings (more common in certain growth forms), the ventral appendages are localized on the under side of the midrib.

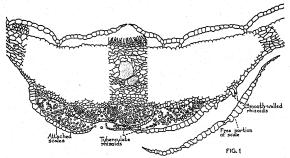


Fig. I. Transverse section of thallus of  ${\it Conocephalum conicum.}$ 

When the relative absorptive capacity of the ventral surfaces was tested by the application of basic dyes such as toluidin blue and neutral red, it was found that the dye was absorbed only by the scales and rhizoids. The dye did not penetrate into the superficial cells of the thallus and was not adsorbed by their walls. The absorptive areas of this plant are the surfaces exposed by the ventral organs which are localized on the midrib.

The under surface of the midrib bears two sets of scales. Each lateral series is formed by the oblique overlapping of individual scales in such a manner that the posterior portion of one is overlapped by the anterior portion of the next scale, two or three members of either series appearing in a given transverse section (fig. 1). Each scale consists of two

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The writer wishes to take this opportunity to express her sincere thanks to Professor R. B. Wylie for suggesting this problem and for his invaluable help and criticisms.

parts: (1) a free anterior portion with a circular appendage, and (2) the elongated portion which is attached by its outer edge to the midrib (fig. 2). The appendage soon withers, and 1-2 cm. back from the growing region only the wedge-shaped, attached portion remains.

The average length of a scale is about 5 mm., the attached portion tapering in width from about 0.5 mm. to a single cell at the posterior end. The surface area of an entire scale is approximately 1.8 sq. mm. The ratio of total scale surface to that of the ventral area of the midrib on which they are borne is 3.8:1. The area of the midrib region is thus increased approximately 380 per cent by means of

scales

A definite localization of the two types of rhizoids for Conocephalum was established by this study. The smooth-walled rhizoids arise from the midrib region covered by the free anterior portion of the scale, and not outside the scale as Cavers (1904) held (fig. 2, 3). They emerge laterally from behind the scales and extend downward to the substrate. The tuberculate rhizoids, on the other hand, arise from that part of the midrib covered by the attached portion of the scale and so are forced to lie nearly parallel to the midrib and within the channels formed between the overlapping scales. Since the rhizoid bases stain sharply with safranin, they may be easily distinguished from other cells of the midrib region, and, with the aid of a biobjective binocular, rhizoidal counts on measured areas of the midrib are readily made.

The total number of rhizoids per centimeter of midrib length ranged from 673 to 1102, with an average of 875, as determined by counts on ten samples from six different thalli. For the plant as a whole, smooth and tuberculate rhizoids are present on the midrib in about equal numbers. Although casual observation suggests that smooth rhizoids have a greater diameter, measurements show that the two types are equal in size, with an average diameter of 15.9 microns. However, they differ greatly in length; the average length of the smooth rhizoids is 6.9 mm., while that of the tuberculates is 16.48 mm. Since the two types occur in equal numbers, the mean rhizoidal length of 11.69 mm. was used in all calculations relating to these appendages. The superficial area of an average rhizoid is 583,915 sq. microns or 0.584 sq. mm. With an average of 87.5 rhizoids per square millimeter of ventral surface of the midrib, the ratio of rhizoidal area to the superficial midrib area on which they are borne is 51:1. The superficial area of the ventral midrib region, therefore, is increased 5100 per cent by rhizoids and 380 per cent by scales, or 5480 per cent by both sets of appendages.

[The Journal for May (26: 271-351) was issued June 7, 1939.] AMERICAN JOURNAL OF BOTANY, VOL. 26, No. 6, JUNE, 1939. While such absorptive areas are important, of perhaps greater significance is the relation of these appendages to the formation of a capillary system outside the plant. The external conduction of water along the ventral midrib may be demonstrated by applying a small amount of aqueous dye to the end of a thallus and watching its spread. Movement of the solution can easily be detected by coloration of absorptive surfaces of the scales and rhizoids as well as by the presence of dye in the interstices between these ventral organs. Conduction from base to apex of an average thallus requires only twenty to thirty seconds, due to rapid movement of liquid in the capillary spaces between the ventral surfaces.

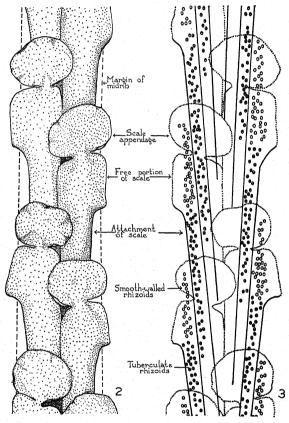


Fig. 2-3.—Fig. 2. External view of scales of Conocepha lum.—Fig. 3. Mirror image of fig. 2 showing insertion of scales and rhizoids.

Discussion.—The ventral specializations of the Marchantiaceae in relation to the distribution of external water, according to Kamerling (1897), were first emphasized by Kny (1890), who suggested the possibility of conduction within the wick-like rhizoidal fascicles. Cavers (1904) speaks of the retention of water within the narrow spaces between scales and rhizoids of Conocephalum conicum (Fegatella conica [L.] Corda). The added possibility of conduction was recognized by Bolleter (1905), who says of the rhizoids: "Indem sie stets dichte Bündel bilden, vermögen sie übrigens Wasser auch

kapillar zwischen sich festzuhalten und zu leiten, eine Wirkung, die durch die Schuppe, in deren Achsel sie entspringen, erhöht wird." A more detailed investigation of the external conduction of water was reported by Bowen (1935) for Fimbriaria blumeana Gottsche. She demonstrated the external conduction of dyes in the narrow interstices between scales and thallus but did not discuss the arrangement and surface relations of its ventral organs.

Concerning water absorption by the thalloid liverworts, there are the following references in Verdoorn's "Manual of Bryology" (1932). Garjeanne (p. 212) states that "in general, water is absorbed by thallus or leaves directly, this being one of the characteristics of the bryophytes." Buch (p. 67) says, "Die Wasseraufnahme durch die Rhizoiden ist eigentlich nur für die Marchantiaceae wichtig, deren Oberfläche ja meist nicht benetzbar ist." Czaja (1936), in testing the alkaline-membrane effect of absorptive tissues, found for Marchantia polymorpha L. that only the cell walls of rhizoids colored with toluidin blue; thallus cells remained unstained and therefore were regarded to be non-absorptive.

For Conocephalum conicum, my tests, using similar methods, show that absorption is limited to the ventral appendages and that the ventral cells of the thallus are non-absorptive. In contrast to Marchantia, absorptive organs of Conocephalum are localized on the ventral midrib rather than distributed over the entire surface of the thallus. For this reason, to the problem of absorption is added that of water distribution, in order that it may be made available to the absorptive surfaces of the whole plant.

Analysis of the midrib appendages of Conocephalum shows that the rapid movement of water along the thallus is greatly favored by the arrangement of the ventral organs. A complex capillary system is formed by the overarching and overlapping scales and the inclosed rhizoids. Since the tuberculate rhizoids arise under the scales, they are prevented from growing downward and are forced into a longitudinal position nearly parallel to each other beneath the several scales of each lateral series. These individual rhizoidal strands, with 40–100 rhizoids each, converge, forming a central capillary system which is continuous for the length of the thallus.

The smooth-walled rhizoids, which emerge laterally from beneath the free anterior portion of the scale, function in anchorage and for direct absorption. They may also serve as temporary lateral branches of the capillary system and supply water to the absorptive surfaces of the plant.

This external capillary retention and conduction of water in Conocephalum conicum may occur in the narrow interstices (1) between tuberculate rhizoids, (2) between rhizoids and their inclosing scales, (3) between rhizoids and the ventral surface of the midrib, (4) between smooth-walled rhizoids,

and (5) between all ventral surfaces and the substrate. The potency of this system for capillary conduction is suggested by the 5480 per cent increase in superficial area of the midrib region through its appendages. When the system is supplied with water, the loose arrangement of the ventral organs permits readjustments which make possible the maximum water retention; with diminishing water supply the surfaces may be brought closer together, thereby adjusting their spatial relations to capillary movement. By means of their ventral protected position, these ventral specializations make possible maximum water retention as well as increased absorptive surface without a concomitant water loss. External water conduction is of fundamental importance in the water maintenance of Conocephalum conicum.2

#### SUMMARY

In Conocephalum conicum (L.) Dumort. water absorption is limited to the ventral appendages.

<sup>2</sup> Since the submission of this paper for publication, Clee (1939) has reported investigations on water conduction in *Pellia epiphylla*. External conduction of water as a capillary film along the under surface of the thallus was found to be "exceedingly rapid," supplying water to the antheridia, archegonia, and developing sporophytes.

All ventral appendages are localized on the under side of the midrib. The area of this region is increased approximately 380 per cent by its scales and 5100 per cent by its rhizoids—together 5480 per cent.

Each scale consists of (1) a free anterior portion, with a circular appendage, and (2) a wedge-shaped portion which is attached by its outer margin to the

ventral surface of the midrib.

The smooth-walled rhizoids arise from the areas covered by the free anterior portions of the scales and readily achieve soil contact. The tuberculate rhizoids originate beneath the attached portions of the overarching and overlapping scales and are forced to assume a longitudinal position, forming strands beneath each scale and parallel to the midrib.

These rhizoidal strands and their overarching scales constitute a complex capillary system which extends the entire length of the thallus.

Rapid conduction of water along the ventral midrib region by external capillarity makes possible the distribution of water to the entire absorptive area of the plant.

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# THE INFLUENCE OF SOIL NUTRIENTS ON DROUGHT RESISTANCE OF TWO-YEAR-OLD RED PINE <sup>1</sup>

Hardy L. Shirley and Lloyd J. Meuli

Observations during the last nine years indicate that droughts of sufficient intensity to cause losses to newly established forest plantations are the rule rather than the exception in the Lake States. In only one of these years did soils on the Chippewa National Forest, Minnesota, fail to dry

<sup>1</sup> Received for publication February 20, 1939.

Delivered before a joint meeting of the Society of American Foresters and the Ecological Society of America, at Richmond, Virginia, December 28, 1938.

Assistance in the performance of experiments, and in the analyses of results, was furnished by personnel of the Works Progress Administration. The statistical treatments used were suggested by R. H. Blythe, Jr., who also supervised the analyses. Help with Russian literature was furnished by S. R. Gevorkiantz.

to the wilting coefficient in the upper two or more inches. Depletion of moisture is particularly common in sandy soils covered with dense sod or low brush which, because they may readily be furrowed, are the areas most frequently chosen for forest planting. Successful planting of these sites is dependent upon using nursery stock with roots capable of penetrating to moist soil layers, and therefore able to evade drought, or upon using stock specially hardened to endure drought. The first requires stock capable of rapid root growth; the second, stock capable of resisting desiccation. Since the degree to which either quality can be induced is limited, best results are to be expected if

the stock possesses both of these characteristics.

Experiments performed during the past six years have shown that drought resistance can be induced in young conifers by exposing them during the growing season to periodic soil dryness and, furthermore, that resistance induced in this manner persists in part during the following season (Shirley and Meuli, 1939). These results were definite and convincing and in general agreement with similar tests on cereal plants reported by Russian workers.

The technique of testing drought resistance has not yet been standardized to the degree that technique for testing cold resistance has. It is not surprising, therefore, to find conflicting interpretations reported in the few papers which have appeared on the influence of mineral nutrients on drought resistance. For instance, using different species, different fertilizing techniques and different methods of estimating drought resistance, Dexter (1937) reports that fertilizers reduce drought resistance; Tumanow (1930), that fertilizers have no effect on drought resistance; and Semakin (1936, 1938), that fertilizers improve drought resistance. Auchter (1932) recommended application of fertilizers, particularly of nitrogenous fertilizers to

drought-injured orchard trees.

Specifically, Dexter (1937) found that rhizomes of quack grass dug from plots heavily fertilized with ammonium sulphate and sprouted in a germinator were more severely injured by exposure to drying for 8, 18, and 48 hours than rhizomes similarly treated but from unfertilized plots. Little difference in apparent drought resistance existed between fertilized and unfertilized rhizomes exposed to drying before being sprouted. Tumanow (1930) found that under conditions of moderate, pronounced, and maximum wilting, the water deficit in leaves and stems, the loss of leaf tissue on drying, and the yield in comparison with unwilted plants of oats and millet were unaffected by applying a fertilizer containing nitrogen, phosphorus, potassium, and sulphur to the light sandy soil in which the plants were grown. From this he concluded that drought resistance is little influenced by soil fertility. Semakin (1936) found that plants can be hardened against drought by exposing them to soil solutions of high concentration. In later experiments Semakin (1938) tested the effect of nitrogen, phosphorus, and potassium applied singly and the three together upon the behavior of barley and grape plants exposed for 6 to 10 days to soil dryness. Drought resistance, as determined by resistance to wilting, by ability to exhaust soil moisture, by osmotic concentration in leaves and stems, by resistance to loss in dry weight upon wilting, and by photosynthetic rate of plants in dry soil, was improved by applications of these mineral nutrients, particularly by applications of phosphorus and of phosphorus in combination with nitrogen and potassium. The nutrients were applied in relatively large amounts, 0.1 to 0.6 grams per liter of sand or soil. The concentrations resulting in maximum drought resistance caused a reduction in growth of barley, but not of grapes. Udolskaja (1934) found that nitrogenous fertilizers lowered the yield of wheat exposed to drought, whereas phosphate fertilizers increased the yield, increased the rate of photosynthesis, and increased the water-retaining capacity of the plant tissue above that of unfertilized plants.

Exploratory studies carried out by the writers in 1933 indicated that drought resistance of conifers was influenced by fertilizing practice. This lead was followed up in 1938 by two experiments designed to test the effects on drought resistance of different concentrations of nitrogen, and of phosphorus and potassium in the nutrient media. Red pine (Pinus resinosa) seedlings, grown for one year in the United States Forest Service nursery at Cass Lake, Minnesota, were used as experimental material.

Sand cultures.—In the first experiment, performed in a greenhouse at St. Paul, seedlings were potted in one-gallon, glazed jars filled with clean silica sand.<sup>2</sup> Analysis showed this sand to contain no available nitrogen and only a trace of available

phosphate.

The following basic nutrient solution was added to all cultures: MgSO<sub>4</sub> · 7H<sub>2</sub>O, 0.055 gram per liter; CaCl<sub>2</sub>, 0.107 gram per liter; H<sub>3</sub>BO<sub>3</sub>, MnSO<sub>4</sub> · 4H<sub>2</sub>O, and FeSO<sub>4</sub> · 7H<sub>2</sub>O, each 0.003 gram per liter. Nitrogen in the form of (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> was added at the rate of 0, 0.245, and 0.49 gram per liter; potassium and phosphorus as KH<sub>2</sub>PO<sub>4</sub> at 0, 0.29, and 0.58 gram per liter. The experiment was factorially designed having all possible combinations of the three levels of nitrogen with the three levels of phosphate (KH<sub>2</sub>PO<sub>4</sub>). To each of these, two watering régimes were added, making a total of 18 individual treatments. Four jars containing 7 seedlings each formed a unit for treatment. The total number of plants involved was 504.

The first nutrient treatment was applied on May 23, two days after potting was completed. Nutrients were applied once weekly, at one-half strength for the first month and at full strength thereafter. At each application sufficient solution was added to saturate the sand; the excess drained off through a hole at the bottom of the jar. Distilled water was added every second or third day until June 28. Thereafter, one group was watered to saturation daily or at two-day intervals, the second at threeor four-day intervals. The longer intervals were used in cloudy weather, the shorter ones in fair weather. The variations in moisture content of the sand were not followed. Aeration was ensured by alternate saturation and draining of the sand. In addition the cultures were aerated once with a tire pump. Treatments were continued until September 9 when the plants were lifted for drought tests. The two watering régimes caused no noticeable differences in appearance and no significant differences in survival, dry weight, or drought resistance;

<sup>2</sup> Obtained through the courtesy of the Ford Motor Company from their plant in St. Paul.

Table 1. Dry weight of red pine transplants supplied nitrogen, and potassium and phosphorus at different levels.

Nitrogen levels		Dry weighta by potassium and phosphorus levels				
		$P_0$	P <sub>1</sub>	$P_2$	-	
Symbol	$(\mathrm{NH_4})_2\mathrm{SO_4}$ per liter	$0.0~ m gram \ KH_2PO_4 \ per liter$	$0.29~ m gram\ KH_2PO_4\ per liter$	0.58 gram KH <sub>2</sub> PO <sub>4</sub> per liter		
	Grams	Grams	Grams	Grams		
$N_0$	0.0	1.19	.90	1.14		
$N_1$	0.245	.91	1.58	1.78		
$N_2$	0.490	.65	1.38	1.35		

<sup>&</sup>lt;sup>a</sup> Means of 19 to 28 plants. Differences in dry weight of 0.33 gram are significant at the 0.01 point.

therefore plants from corresponding nutrient treatments from the two watering régimes are hereafter considered to belong to the same treatment.

The total mortality during the 109-day period of nutrient treatment which preceded drought tests was 2 out of 168 plants in the 3 cultures without nitrogen, 6 out of 112 plants in the 2 cultures having nitrogen at single strength plus phosphate, and 106 out of 224 plants in the 4 cultures having nitrogen at double strength and nitrogen at single strength without phosphate. The color of plants receiving no nitrogen was yellow green, whereas that of those receiving nitrogen in single or double strength solutions was dark green. As to size, plants without nitrogen or without phosphorus and potassium were smaller, had shorter needles and more fibrous roots than those receiving all three nutrient elements. The average dry weights by nutrient levels, determined after the drought tests were completed, showed the plants in the N<sub>1</sub>P<sub>2</sub> group to be the largest (table 1).

The seedlings were repotted individually in cans containing sand at 5 per cent moisture content and exposed in the 1936 drought machine (Shirley and Meuli, 1939) at an air temperature of 37°C. and at a relative humidity of 25 to 30 per cent. After three days, the plants were removed, freed of soil,

the top and root of each weighed fresh, then dried for 48 hours at 105°C. and reweighed. The percentage moisture content after 3 days' exposure was used as an index of relative drought resistance. Experience has shown this to be closely correlated with the total length of time plants will survive in the machine. Moisture content varied from 29 to 130 per cent, depending on treatment. Plants having only 29 per cent moisture were obviously dead; those having 130 per cent were to all appearances in good condition. The moisture percentage, irrespective of treatment, was found to be inversely correlated with total dry weight, the regression coefficient being -20. To separate the size effect from the nutrient effect, the data were analyzed by the covariance method. This reduced the error and the differences between treatments but caused no significant change in their order. Differences due to nutrient levels were found to be significant beyond the .01 point. The average moisture contents by treatments, after adjusting for size, are shown in table 2.

Cultures without nitrogen or without potassium and phosphorus produced more drought-resistant plants than those in which all three of these elements were present. Additions of potassium and phosphorus improved resistance if no nitrogen was

Table 2. Moisture content<sup>a</sup> after three days' exposure to artificial drought of red pine transplants supplied nitrogen, and potassium and phosphorus at different levels.

 Nitrogen levels		Moisture content <sup>b</sup> by potassium and phosphorus levels						
		$P_0$	$\mathbf{P_1}$	$P_2$				
Symbol	$(\mathrm{NH_4})_2\mathrm{SO_4}$ per liter	0.0 gram KH <sub>2</sub> PO <sub>4</sub> per liter	$0.29~ m gram \ KH_2PO_4 \ per liter$	$0.58~ m gram\ KH_2PO_4\ per liter$				
	Grams	Percentage	Percentage	Percentage				
$egin{array}{c} N_0 \ N_1 \ N_2 \end{array}$	0.0 0.245 0.490	92 75 98	123 36 41	123 57 42				

<sup>&</sup>lt;sup>a</sup> Adjusted for plants equal in weight. <sup>b</sup> Means of 19 to 28 plants. Differences in moisture content of 21 per cent are significant at the 0.01 point.

present. With nitrogen supplied at single strength, double-strength phosphate solutions produced more resistant plants than single-strength phosphate. In fact, of the 4 cultures having both nitrogen and phosphate, the  $\rm N_1\,P_2$  treatment gave the highest survival during nutrient treatment, the largest plants and the highest moisture content at the end of the drought test. The  $\rm N_2\,P_0$  culture gave the lowest survival of all nutrient treatments and produced the smallest plants. These, however, were more drought resistant than the  $\rm N_1\,P_2$  group.

Top-root ratios were calculated but showed only a rough correlation with drought resistance. They were low in the  $N_0P_1$  and  $N_0P_2$  groups, intermediate in the  $N_0P_0$  and  $N_1P_0$  groups, and high in the

remaining groups.

NURSERY CULTURES.—The second experiment was carried out in the nursery at Cass Lake, Minnesota. The soil on an area approximately 15×45 feet was removed to a depth of 16 inches, thoroughly washed in a concrete mixer, and rinsed in a train of two sluice boxes. The coarse sedimented sand was returned to the nursery bed. This consisted of 4 per cent fine gravel, 17 per cent coarse sand, 33 per cent medium sand, 42 per cent fine sand, and 4 per cent very fine sand. Nitrate nitrogen was present at the rate of 2 pounds per acre, available phosphate at the rate of 50 pounds per acre. The area was divided into 18 beds 5×6 feet each, into which red pine seedlings were transplanted at a spacing of  $2\times 6$ inches. The experiment was arranged factorially having all possible combinations of ammonium sulphate at 0, 0.5, and 1.0 gram per square foot, with 20 per cent commercial superphosphate at 0, 1.67, and 3.33 grams per square foot, with frequent and infrequent watering, making a total of 18 individual treatments as in the greenhouse cultures. Ammonium sulphate was applied in solution; superphosphate was dusted on the surface and hoed into the soil. Both fertilizers were applied biweekly at one-half concentration from May 9 to June 18, and monthly at full concentration thereafter. The beds were thoroughly watered following each application. Differential watering was begun on June 18; thereafter one set of beds was watered daily, the other at intervals of 3 to 6 days depending on the weather. Soil moisture in daily-watered beds varied from 12 to 16 per cent; in infrequently-watered beds, from 5 to 16 per cent.

By the end of the summer, plants which had received no nitrogen were distinctly chlorotic; those receiving 0.5 gram per square foot had light green foliage; those receiving 1 gram per square foot had dark green foliage. Of the plants receiving nitrogen, those watered daily were lighter in color and smaller in size than corresponding plants watered infrequently. This indicates that daily watering must have caused leaching of the readily soluble ammonium sulphate from the sandy soil. No differences due to phosphate levels were discernible, an indication that the soil contained enough to support growth of red pine seedlings.

Five tests of drought resistance were made during the course of the summer in a new drought machine similar in construction to the 1936 model. Ten plants from each of the 18 treatments were used in each test. The seedlings were lifted, weighed fresh, potted in tin cans containing sand at 5 per cent moisture content, and placed in the machine where air temperature was held at 37°C, and relative humidity between 25 and 35 per cent. Each plant remained in the machine until dead, that is until its moisture content was reduced to approximately 50 per cent, as determined by shriveling of needles and increase in their rigidity. Plants so desiccated have invariably failed to recover when watered. The number of days survived is used as a criterion of drought resistance. Nonresistant plants have been found to die on the second or third day when available soil moisture approaches exhaustion; moderately resistant plants live 4 to 7 days, and highly resistant plants 8 days or longer.

The effect of fertilizers on drought resistance was clearly evident as early as July 11 when the first test was made. The magnitude of this effect became greater as the season progressed, and as the drought resistance of plants from all treatments improved. The fifth test, begun on September 12, gave the most striking results. In these tests also small plants appeared to be more drought resistant than large ones. The data were therefore analyzed by the covariance method to separate the size effect from the treatment effect. Again this analysis reduced the error and the differences between nutrient treatments but did not change their order. There was a tendency for drought resistance to decrease with increasing phosphate supply except in the minus nitrogen beds where additions of phosphate apparently improved drought resistance. Since these differences were not significant in either case, the three phosphate treatments are considered as though combined in the discussion which follows. Differences due to nitrogen supply and to the interaction of nitrogen supply and water supply were found to be significant at the .01 point (table 3). Ammonium sulphate reduced drought resistance in direct relation to the amount supplied. Infrequent watering definitely improved the resistance of plants deprived of nitrogen and probably of those supplied nitrogen at single strength; it had a tendency to decrease the resistance of plants supplied nitrogen at double strength. This is believed to be a reaction to higher nitrogen concentration in the infrequently watered, and therefore less leached, bed.

Discussion.—In both experiments drought resistance was found to be markedly reduced by applications of nitrogen. The effect proved to be the same irrespective of whether nitrogen was present only in sufficient concentration to result in optimum growth as in the nursery test or present in concentrations high enough to be somewhat toxic as in the greenhouse test. Only in the case of the  $N_2P_0$  treatment from the greenhouse, which was so toxic that only 23 out of 56 plants survived the period of nu-

Table 3. Average fresh weight and survival in drought machine of red pine transplants in relation to nitrogen and moisture supply.

Nitrogen supply		Fresh weight <sup>b</sup>		Sı		
Symbol	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> applied monthly per square foot	Watered daily	Watered every 3–6 days	Watered daily	Watered every 3-6 days	
	Grams	Grams	Grams	Days	Days	
$N_0$	0.0	2.39	2.52	7.65	8.09	
$N_1$	0.5	3.69	4.85	7.01	7.20	
$N_2$	1.0	4.48	6.79	6.53	6.41	

<sup>&</sup>lt;sup>a</sup> Adjusted for plants equal in weight. Differences in survival of .33 day are significant at the .01 point. <sup>b</sup> Means of 30 plants.

trient treatment, did high concentration of nutrients increase drought resistance over that of corresponding plants from moderately fertilized cultures.

The fact that phosphate had a much more pronounced influence on drought resistance in the greenhouse cultures than in the nursery cultures is probably due to adequate phosphate being present in all nursery beds. Wilde (1938) reports that 50 pounds of available phosphate per acre, the amount found to be present in the washed nursery soil, is adequate for good growth of red pine in natural stands and by inference for red pine nursery stock also.

Until a more complete series of tests are available, in which internal nutrient concentrations are known as well as external supply, it seems presumptive to speculate on the mechanism involved. Emmert (1936) reports that soil dryness causes an accumulation of nitrates in tomato plants and a decrease in their phosphorus content. Possibly the lowered resistance of infrequently watered plants in the N<sub>1</sub> and N<sub>2</sub> nursery series may have been due in part to an increase in internal nitrogen concentration, though it seems more likely that it was due chiefly to the higher external concentration resulting from less leaching in the infrequently watered beds. It may also be pointed out that high nitrogen content has been found to reduce cold resistance (Dexter, 1935). No tests of cold resistance were made of the plant material used in these drought tests. It had been determined in preliminary experiments that exposing seedling conifers lifted in midsummer to low temperatures at night did not increase appreciably their resistance to drought. Semakin (1938) found that grape plants receiving extra potassium, though less drought resistant, were more cold resistant than those receiving extra phosphorus. The writers believe that it is not wise to press the analogy between drought resistance and cold resistance too closely, because as Maximow (1929) points out, there are plants cold resistant but not drought resistant, and others drought resistant but not cold resistant.

These experiments, and also those of Semakin (1938) indicate that drought resistance is influenced by the balance between nutrient elements as

well as by the concentration of nitrogen alone. The production of plants best able to survive natural droughts, such as occur in the field, is probably dependent upon maintaining an optimum combination of several nutrient elements.

Though nitrogen starvation increases the capacity to endure drought, it decreases growth. Large-sized plants with efficient leaf systems are necessary to supply food for rapid root penetration. This determines capacity to evade drought, which is often of greater importance than capacity to endure drought. Application of these findings to nursery practice should therefore be deferred until a schedule can be prescribed by which normal-sized and at the same time drought-resistant plants can be produced.

## SUMMARY

One-year-old red pine seedlings were cultured during the second growing season in pots and nursery beds supplied varying amounts of nitrogen, phosphate, and water. At the end of this period they were tested by exposure to controlled drought.

Increase in nitrogen content consistently resulted in a decrease in drought resistance.

Nursery-grown plants watered daily were less resistant than those watered at 3-day intervals if nitrogen was absent. With nitrogen present this effect was obscured because heavy watering resulted in leaching of nitrogen.

Phosphate supply also influenced drought resistance but its effect varied with nitrogen concentration. With no nitrogen present an increase in phosphate increased drought resistance. With nitrogen present this effect tended to persist, but the optimum combination of nitrogen and phosphate was not determined.

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# A LOW COST CHAMBER FOR PHYTOHORMONE TESTS <sup>1</sup>

G. S. Avery, Jr., H. B. Creighton, and C. W. Hock

Because of the expense involved in providing the necessary constant-temperature constant-humidity rooms for carrying on quantitative research on plant growth hormones, studies in this field have been practicable in only a few institutions. Teachers of plant physiology desiring to offer quantitative phytohormone work to their students also have been handicapped by the lack of facilities. Even when properly controlled rooms are available, it is impossible to have many students working in the same room, because of the difficulty in controlling temperature, humidity, and carbon dioxide content of a room in which several persons are working.

The miniature control chamber described below, which may be constructed for fifty dollars or less, provides the necessary conditions as regards humidity and may be used in any darkroom with controlled temperature. If a constant-temperature room is not available, the chamber may be put in a temperature-regulated fiberboard case.

Description of chamber and inner rotating table.—The chamber consists of a circular sheet metal compartment 30 inches in diameter, tightly fitted with a removable cover (fig. 1, 2, 3). Attached to one side of the compartment is a small glass-covered vestibule which opens into the chamber by a removable door. The operator works on the test plants by putting his arms through holes in the front of the vestibule. The glass cover of the vestibule is set in a hinged frame, so that it may be opened as a door; this provides additional means of access to the chamber, via the vestibule, and may be used for larger objects whenever it is not desirable to remove the cover of the chamber.

The rotating table provides space for eight or nine dozen test plants (water culture), all of which may be reached readily through the small door be
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tween the chamber and the operating vestibule. The rotating table with its supporting frame may be removed at will (fig. 1).

Specifications for construction.—The sheet metal outer chamber is of 24 gauge galvanized iron, the bottom double-seamed and soldered so as to be water tight. The vestibule is connected with the humid chamber by a soldered lap joint. The removable cover for the humid chamber is double-seamed and hemmed on the edge (snug fit). The door above the vestibule consists of a galvanized iron frame around double thick window glass (fig. 1, 2).

The supporting framework for the rotating table is made entirely of galvanized band iron  $1'' \times 1/8''$ . Top and bottom cross pieces are bent down at the ends and fastened to uprights with brass nuts and bolts. For support, bolts at the center bottom of the supporting framework extend to bottom of outer chamber (fig. 1, 3).

The rotating table has cross supports made of  $1'' \times \frac{1}{8}''$  galvanized angle iron cut back 1'' on the side and the overhanging 1'' top bent down. The outer hoop is of  $1'' \times \frac{1}{8}''$  galvanized band iron fastened to the bent-over ends of the angle iron by brass screws. The cross supports are covered with  $\frac{3}{8}''$  mesh hardware cloth soldered on same and extending to the hoop where it is also soldered (fig. 1, 3).

The axil on which the rotating table turns is made of  $\frac{3}{8}$ " brass rod, long enough to extend through top cover (for revolving from the outside, if necessary). The rod is turned down to  $\frac{3}{16}$ " for a distance of  $\frac{1}{2}$ " at the lower end, in order to form a bearing for the rotating table in the supporting framework (fig. 3).

The humid chamber, rotating table, etc., may be paraffined to prevent corrosion of the metal.

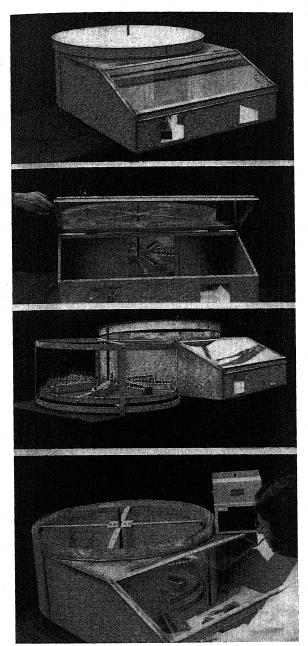


Fig. 1. Photographs of control chamber. Top: with lid on, showing operating vestibule and door leading from vestibule into humid chamber. Upper center: with lid off and hinged glass cover of operating vestibule raised. Lower center: rotating table with supporting framework, removed from control chamber; on rotating table note arrangement of zinc trays with water, and adjacent racks of Avena test plants. Bottom: showing position of operator when decapitating Avena test plants in vestibule. The door between vestibule and humid chamber is closed except when removing (or replacing) test plants from humid chamber.

TEMPERATURE CONTROL OF THE CHAMBER.—The chamber should be used preferably in a darkroom

in which the temperature is controlled at 78-79°F. Wherever a steady supply of steam is available, uniform heating of the darkroom can be accomplished with any existing radiator by installing thermostat-controlled electrically operated steam supply valves. The amount of radiation needed depends upon the heat loss from the room.

If electrical heating is needed or desired, the necessary number of electrical heating units can be readily installed to maintain the room at the desired temperature. These heating units, if controlled by a standard thermostat and relays, should regulate the temperature of the room to within + or - one degree F. No definite specifications for size of the electrical heating units can be given unless the size and construction of the room are known. To be heated economically by electricity, the room should be insulated.

If a fiberboard case (such as that illustrated in fig. 4) is constructed around the chamber, it may have the same electrical heating arrangement as described for a darkroom.

HUMIDITY CONTROL.—The volume of the chamber is such that eight or nine zinc trays  $1\frac{1}{4}"\times 1\frac{1}{4}"\times 8\frac{1}{2}"$ , approximately three-fourths full of water, will provide a relative humidity of 86–88 per cent at 78–79°F. This is the optimum humidity and temperature reported for "standard" Avena tests. When setting up the chamber in preparation for tests, the zinc trays (paraffin coated) should be filled with water already at a temperature of 78–79°. If this is done, the relative humidity in the chamber climbs to 88 per cent within 8 hours (fig. 5), whereas, if started with cold water, the humidity climbs slowly over a period of 15 hours. Figure 5 shows full thermohumidigraph records of conditions in the chamber during several tests.

In order to maintain a constant humidity in the operating vestibule while decapitating, applying blocks, and removing for photographing, paper towels thoroughly moistened are placed on the floor of the operating vestibule an hour or so before tests are started. If the towels show any sign of drying out during these operations, or later during photographing, they should be re-moistened.

As a precaution against any water being picked up by the racks of test plants during decapitation, etc., a dry block of wood large enough to act as a stand for a rack of test plants is placed in the vestibule during decapitation, etc. For the humidity to remain at known levels, as indicated in figure 5, the racks should be dry both when the experiment is set up and during subsequent operations. Any water clinging to the racks increases the evaporating surface, causing a rise in humidity within the chamber. Also, as a precaution against unduly increasing the humidity within the chamber, care must be exercised not to spill any water when the trays are placed on or removed from the rotating table. Such precautions are simple in practice.

ILLUMINATION.—Various types of illumination for the chamber may be used. The main points to

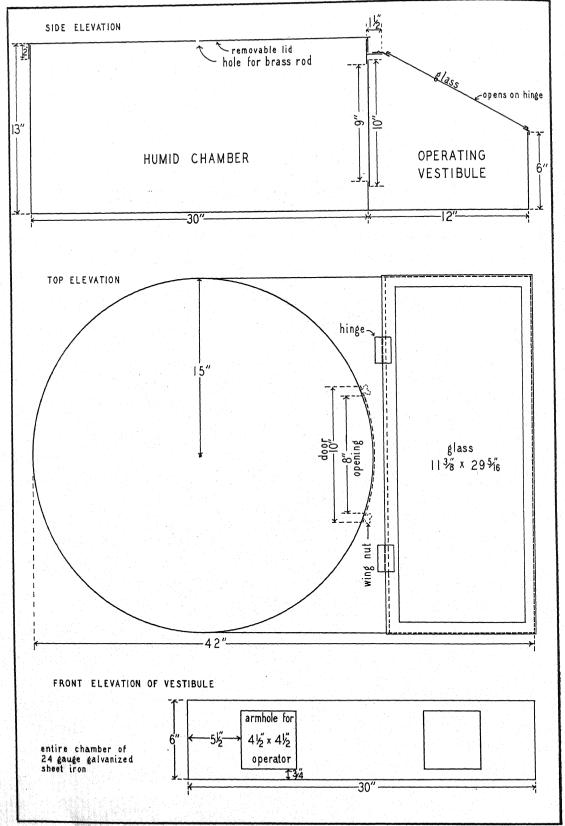


Fig. 2. Working drawings of top and side elevation of control chamber.

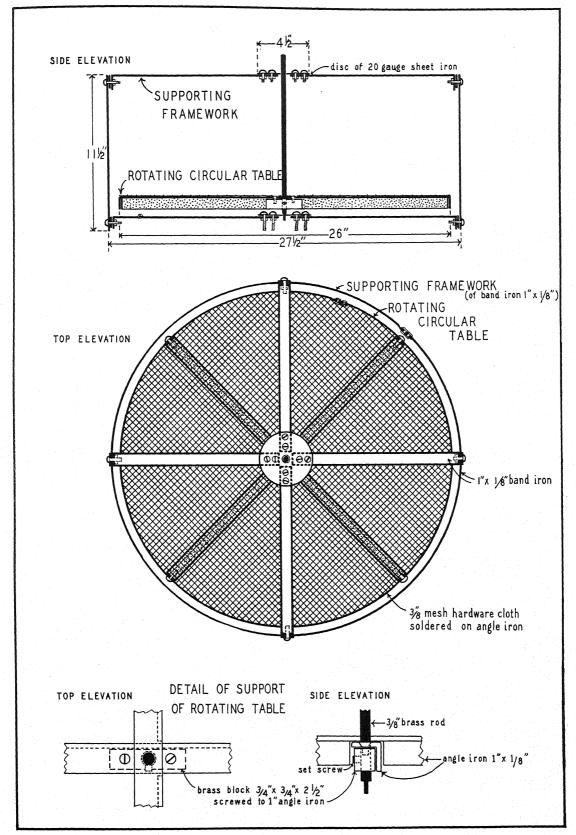


Fig. 3. Working drawings of top and side elevation of rotating table and its supporting framework.

be considered are, first, that the light be so placed that reflections from the vestibule window do not impair vision, and, secondly, that the temperature of the chamber is not raised. A simple source of light is an Eastman Safelight Lamp, size no. 2, in which has been placed a Corning Glass Co. red filter no. 246. Any other type of lamp providing phototropically inactive light is satisfactory, as long as the temperature in the chamber is not affected. For this latter reason and for best illumination, the lamp may be set directly upon the operating vestibule, rather than upon the chamber.

RESULTS OF TESTS WITH INDOLE ACETIC ACID.-The data given are the result of comparative tests in the control chamber and in the usual controlledtemperature controlled-humidity testing room. The accompanying table illustrates the results of tests on more than 100 doz. test plants, using both the "standard" method of Went (1928) and Skoog's "deseeded" method (1937).

The somewhat greater curvatures obtained in the regular test room may possibly be attributed to the

Table 1. Avena coleoptile tests in the control chamber and in the usual test room, using 3-indole acetic acid made up in 1.5 per cent agar, in the concentrations indicated. Results are given in "degrees curvature" of the test coleoptiles, each figure being the average of twenty-four coleoptiles.

"Deseeded" method (Skoog) (photo-

graphed 5 hours after applica-

	tion of agar blocks)						
	0.01 mg. per liter		0.02 mg. per liter				
Date	Control chamber	Test	Control chamber	Test room			
January 23		9.0	19.4	19.9			
24	9.7	9.5	18.9	18.9			
February 1	6.9	8.9	14.3	21.0			
10		10.3		~1.0			
21	. 7.3	7.7	18.4	16.1			
22		13.5	21.5	26.8			
24	. 10.3	8.9	18.9	23.3			
Average	. 8.9	9.7	18.6	21.0			

"Standard" method (Went) (photographed 2 hours after application of agar blocks)

	0.02 mg. p	er liter	0.04 mg. per liter			
Date	Control chamber	Test room	Control chamber	Test		
January 30		5.3	9.3	11.3		
31	4.6	7.2	11.4	14.0		
February 1		8.1	8.1	12.2		
10			7.7	10.2		
20		5.7	9.7	9.9		
22			8.0	10.2		
24	4.8	5.5	9.0	8.7		
Average	4.6	6.4	9.0	10.9		

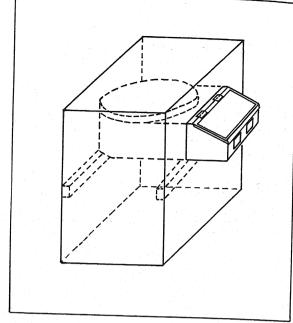


Fig. 4. Diagram of suggested fiberboard case for maintaining constant temperature around control chamber if a constant temperature room is not available.

higher humidity, the test room being, on the average, 5 to 10 per cent more humid than the control chamber. For the week of January 23, however, the relative humidity of the test room was about the same as for the control chamber; the Avena tests were also in close agreement. It is of interest to note that in these tests the curvatures obtained, per unit of indole acetic acid, are about four times greater with the "deseeded" test method than with the "standard" method.

SCHEDULE OF TEST METHODS .- "Deseeded" Avena method (with endosperm removed) (Skoog) .-The following time schedule was chosen as a convenient one:

First day, 9 a.m. Soak hulled oats 2-4 hours, then pour off water and set seeds, embryo side up, about  $\frac{1}{2}$  apart, in rows  $\frac{3}{4}$  apart, on moist filter paper in 150 mm. diameter Petri dishes. After a few hours exposure to light in the laboratory, place in 78-79°F. darkroom (5 or 6 p.m.), and allow to grow undisturbed until the third day.

Third day, 2 or 3 p.m. Set dishes of seedlings at approximately a  $60^{\circ}$  angle so coleoptiles will grow upward, and straight. 8 to 10 p.m. At approximately 60 hours of age, the coleoptiles should be 1.5 to 2 cm. in length. The endosperm is carefully broken off with the fingers. A small tuft of twisted cotton is wound around the region of the removed endosperm, and the seedling pulled carefully into the glass holder. At the time of pulling into the holders, the coleoptiles are so oriented that the broad side is parallel with the rack, hence the agar blocks are subsequently placed over a vascular bundle. After

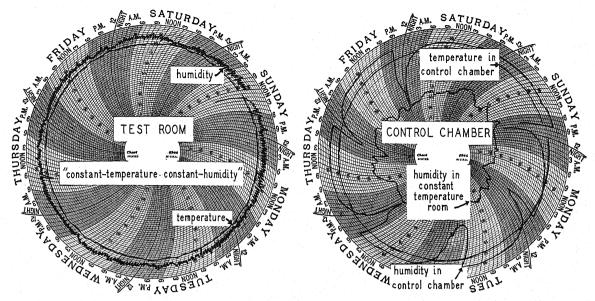


Fig. 5. Thermohumidigraph records of temperature and relative humidity in the control chamber and test room (week of Jan. 23). The humidity record of the room in which the control chamber was housed has been traced on the same recorder sheet with the data from the chamber. In the control chamber each sharp dip in the humidity curve indicates the time at which the lid was removed to set up a new experiment. The zinc trays for the "Monday" experiment were filled with cold tap water; note how slowly the humidity increases under such conditions. In all other experiments the trays were filled with water at a temperature of 78–79°F.

a little experience it is possible for the operator to merely feel the broad vs. the narrow side of the coleoptile and thus orient it quickly and properly. Upon completion of the "deseeding" operation, each rack of test plants is placed with the seedling roots immersed in a tray ¾ full of water (78-79°F.). The "deseeding" is done in the darkroom, outside the control chamber. When 8 or 9 racks of test plants have been "deseeded," they are carefully placed, together with the trays, on the rotating table in the humid chamber. The lid may be removed for this purpose, or they may be inserted through the door between the chamber and the operating vestibule.

Fourth day, 1 to 2 p.m. In the operating vestibule, 17-18 hours after "deseeding," decapitate as in the "standard" method and immediately apply agar blocks to be tested, then replace in humid chamber. Photograph 5 hours later.

"Standard" Avena method (Went).—This test is too well known to require mention of test schedule, but it may be pointed out that the test plants with endosperms still attached, are placed in the holders at the same time that the "deseeded" plants are placed in holders. After decapitation on the fourth day, forty minutes are allowed to elapse before application of agar blocks. Photographs are taken after 2 hours (Went originally stipulated 110 minutes). Schneider and Went (1938) have made available further information on this test.

## SUMMARY

A chamber is described for use in the Avena test for plant growth substances. It permits perfect humidity control if kept in a temperature controlled darkroom and thus obviates expensive humidity control equipment and maintenance expense. The chamber is large enough to care for 8 or 9 racks of Avena seedlings (each holding 12 plants) in water culture. Several control chambers may be housed in a single darkroom. Schedules are given for the "deseeded" and "standard" Avena methods (Skoog and Went) as used in comparative tests in the new control chamber and in the usual constant-temperature constant-humidity test room. The results of such tests on more than 100 dozen test plants are included.

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# GRASS STUDIES. III. ADDITIONAL SOMATIC CHROMOSOME COMPLEMENTS 1

Etlar L. Nielsen

The work reported in this paper is a continuation of the investigations begun by Nielsen and Humphrey (1937). The material used in the study, taken from plants grown in the greenhouse, was fixed in chrom-acetic-formalin, the embedded material sectioned at  $12~\mu$  and stained with iodine-crystal-violet. The plants later were transplanted into the field where they were grown to maturity. The identifications were made with the aid of Hitchcock's (1935) "Manual of Grasses of the United States." The camera lucida drawings were made at a projected magnification of  $1500\times$ ,  $2100\times$ , or  $2600\times$ .

The micro-morphological measurements reported for the nuclear diameters were made upon nuclei in metabolic condition located in the meristematic region of the root tip. Fifty measurements were used to determine the average figure given for each case discussed. These and similar data for the chromosomes are summarized in table 1. Included in this table also are the Arkansas accession numbers, the seed or clonal source, and the original collector and year of collection if known.

FESTUCEAE.—Stahlin (1929) and Avdulov (1928) have reported complements of 42 and 56 chromosomes, respectively, for races of Bromus inermis Leyss. The writer has confirmed Avdulov's observations of 56 chromosomes in somatic tissue and examined material of an additional race of this series with complements of 70 chromosomes. The 56-chromosome race has thicker and shorter chromosomes and smaller nuclei than the 70-chromosome race (fig. 1, 2). Herbarium specimens prepared from the plants from which the cytological material was taken show little morphological difference other than a slight increase in size of the latter race. This is also true of other plants of these same selections now being grown in the grass nursery at this station.

Bromus marginatus Nees is composed of a series of races closely paralleling that of Bromus inermis. This species has previously been reported to have races with complements of 28 by Horton and Beck (1932), of 42 by Nielsen and Humphrey (1937), and of 56 chromosomes by Avdulov (1931). To this series of races has been added an additional one with a complement of 70 (fig. 3). A comparison of the members of the complements of the 42- and 70-chromosome races shows that the former race has thicker chromosomes and smaller nuclei. Unfortunately, no herbarium specimens were prepared of the 42-chromosome strain previously reported so that no gross morphological comparisons can be drawn.

Root tip cells of *Bromus commutatus* Shrad. and *B. secalinus L.* were characterized, respectively, by <sup>1</sup> Received for publication April 3, 1939.

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complements of 56 and 14 chromosomes (fig. 4, 5). Although the diameters of the chromosomes of both species were similar, the lengths of those of the former species varied from  $2.5~\mu$  to  $7.5~\mu$  as compared with  $3.5~\mu$  to  $6.0~\mu$  for the latter. No other significant morphological differences such as constrictions and satellites were detected. Avdulov (1928) had previously reported B. secalinus to have 28 chromosomes, a complement which was subsequently verified by Stahlin (1929) and Nakajima (1931). This new number for this species thus suggests another polyploid series in members of this genus.

Glyceria grandis S. Wats. was found to have 28 chromosomes (fig. 6), mostly about 0.5  $\mu$  in diameter and from 1.5  $\mu$  to 3.5  $\mu$  in length. The individual members of the complements are irregularly rod-like to U-shaped and show no distinguishing morphological characters. The nuclei averaged 8.1  $\mu$  in diameter

Chromosome complements of 40 were observed in Eragrostis curvula (Schrad.) Nees and E. sessilispica Buckl. (fig. 7, 8). Likewise, material from Eragrostis spectabilis (Pursh) Steud., which was previously reported by Humphrey (1937) and by Nielsen and Humphrey (1937) as having 42 chromosomes, has been reexamined by the writer and found to have a complement of 40 (fig. 9), which thus places the basic number of this species in accord with those of other species of Eragrostis herein reported and with those previously reported by Avdulov (1928, 1931) and Hagerup (1932). The complements studied were made up of members characteristically about  $0.3 \mu$  in diameter which ranged from 1.0  $\mu$  to 2.2  $\mu$  in length. Their shape is generally rod-like, and they show no signs of such morphological features as constrictions. The average nuclear diameters were 7.9  $\mu$  for E. curvula, 8.0 μ for E. sessilispica, and 12.0 μ for E. spectabilis.

The Festuceae just reported fall into two groups as shown by these species of Bromus, Glyceria, and Eragrostis. Bromus and Glyceria have rather long, sometimes rod-like, but more often vermiform to U-shaped chromosomes characteristically in multiples of 7; Eragrostis has short, more regularly rod-shaped or slightly curved chromosomes occurring in multiples of 10. Although these observations, and a majority of those recorded for other species of this tribe, indicate these distinct groups, there are some records that multiples of other numbers also occur in the tribe. Avdulov (1928, 1931) reported diploid numbers of 18 in Melica altissima L., M. ciliata L., M. micrantha Boiss. and Hohen., and M. nutans L. Kattermann (1930) found these numbers correct for M. altissima and M. nutans, and Hunter (1934) verified the report of 18 chromosomes in M. altissima.

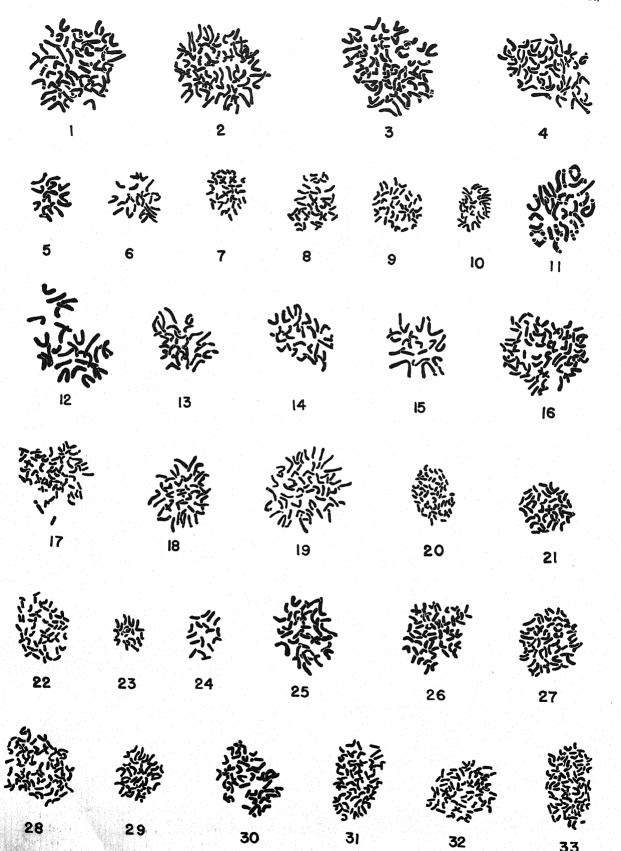
TABLE 1.

Ark.				D:1	Chromosome		Nuclear
Acc. number	Plant name	Seed or clone source	Collector	Dipl. comp.		Length	
FES	TUCEAE:						
372.155	Bromus marginatus	Unknown	Unknown	42	.7	3.0 - 6.0	11.7
110	Bromus marginatus	Between Cheyenne and Laramie, Wyo.	Unknown	70	.5	3.0-6.0	13.5
107	Bromus inermis	Lincoln, Neb.	Fults (1935)	56	.6	1.5 - 4.0	8.3
105	Bromus inermis	Lincoln, Neb.	Lyness	70	.5	2.0 - 7.5	9.7
93	Bromus commutatus	Albion, Neb.	Unknown	56	1.0	2.5 - 7.5	11.4
90	Bromus secalinus	Colorado and Utah (?)	Maguire & Piranian (1935)	14	1.0	3.5-6.0	9.2
276	Glyceria grandis	Conifer, Colo.	Hanson & Conrad (1934)	28	.5	1.5-3.5	8.1
884	Eragrostis curvula	Kimberly, Union of South Africa	Miss M. Wilman (1927)	40	.3	1.0-1.5	7.9
249	Eragrostis sessilispica	Caddo Co., Okla.	Unknown	40	.3	1.0-2.2	8.0
	Eragrostis spectabilis	Unknown	Unknown	40	.3	1.0-1.7	12.0
~~							. 7
233 233	DRDEAE: Elymus canadensis var. robustus	Boone, Iowa	Fults (1934)	28	.8	2.5-7.0	11.5
232	Elymus Macounii	Greybull, Wyo.	Porter (1934)	28	.5	2.0-5.0	10.2
235	Elymus sibericus	S. C. S. Lincoln, Neb.	Unknown	28	.8	3.0-7.0	7.8
173	Calamagrostis epigeios	Hailar, Manchuria	McMillan & Stephens	28	.5	2.0-4.0	9.7
45	Agrostis exarata	Palouse, Wash.	Unknown	28	.5	1.5-3.5	8.0
439	Sporobolus cyptandrus	Gallup, N. M.	Hanson & Conrad (1934)	18	.7	2.5-6.0	7.8
14	Sporobolus heterolepis	O'Neil, Neb.	Nielsen (1935)	72	.5	1.0-3.0	10.5
466	Stipa pulchra	Santa Paula, Calif.	Unknown	66	.5	1.0-3.0	
463	Stipa columbiana	Poorman Creek, Helena, Mont.	Nelson (1934)	44	.5	1.5-3.5	
	Muhlenbergia pungens	, Neb.	Fults (1934?)	60	.5	1.5-4.0	9.2
24	ILORIDEAE: Buchloë dactyloides	O'Neil, Neb.	Nielsen (1935)	56	.5	.7–2.0	10.1
PA 610	NICEAE: Paspalum Urvillei	Rosston, Ark.	Nielsen (1936)	40	.5	1.0-2.5	7.9
604	Paspalum Urvillei	Magnolia, Ark.	Nielsen (1936)	60	.5	1.0-2.0	8.1
612	Panicum anceps	Rosston, Ark.	Nielsen (1936)	18	.5	1.5-3.5	
598	Panicum Lindheimeri	Newport, Ark.	Nielsen (1936)	18	.5	1.5-2.5	
337	Panicum texanum	Buville, Tex.	Unknown	36	.7	1.5-4.0	
	NDROPOGONEAE:	Th	Nielgen (1996)	60	.7	2.0-4.0	9.0
576	Andropogon furcatus	Fayetteville, Ark.	Nielsen (1936)	60			
35	Andropogon Hallii	Inez, Neb.	Nielsen (1935)	60 70	.5	1.0-3.0 1.0-3.0	
33	Andropogon Hallii	Inez, Neb.	Nielsen (1935)		.5		
860	Andropogon saccharoides	Hope, Ark.	Nielsen (1936)	60	.5	1.0-4.0	
859	Andropogon saccharoides	Hope, Ark.	Nielsen (1936)	70	.5	1.5-3.5	
856	Andropogon scoparius	Rosebud, Ark.	Nielsen (1936)	40	.5	2.5-4.5	
848	Andropogon scoparius	Damascus, Ark.	Nielsen (1936)	40	.7	2.5-7.5	
130	Andropogon perforatus	San Antonio, Tex.	Mott (?) (1934)	80	.5	1.5-4.0	9.7

Hordeae.—The complements of Elymus canadensis var. robustus (Scribn. and Smith) Mackenz. and Bush, E. Macounii Vasey, and E. sibiricus L. each have 28 members (fig. 10, 11, 12). All appear alike, being long and often vermiform to U-shaped in outline. Those of E. Macounii are mostly about 0.5  $\mu$  in diameter as compared with 0.8  $\mu$  in the other species here considered. They range from 2.0  $\mu$  to 7.0  $\mu$  in length. Average nuclear diameters for the species studied varied from 7.8  $\mu$  to 11.5  $\mu$ .

These observations and many of those recorded in literature for other species of this tribe indicate that 7 is the prevalent basic number of the group. Deviations from this number have been found by Avdulov (1931), who reported a complement of about 36 chromosomes in Lepturus incurvatus Trin. (Pholiurus incurvatus (L.) Schinz. and Thell.) and one of 26 in Nardus stricta L.

AGROSTIDEAE.—Avdulov (1931) estimated the chromosome number in *Calamagrostis epigeios* (L.) Roth to be 70. The present writer found this species also may have the tetraploid number of 28 (fig. 13). One pair of chromosomes has well-defined submedial constrictions. The members of the com-



plement average slightly less than 0.5  $\mu$  in diameter and vary in length from 2.0  $\mu$  to 4.0  $\mu$ . The average nuclear diameter is 9.7  $\mu$ .

Agrostis exarata Trin. has 28 chromosomes (fig. 14)  $0.5~\mu$  in diameter varying from  $1.5~\mu$  to  $3.5~\mu$  in length. They are either rod-like to U-shaped in form, with none of the members showing distinguishing structural characters. The nuclear diameters

average 8.0 μ.

Sporobolus cryptandrus (Torr.) A. Gray was found to be a diploid species with 18 chromosomes, and S. heterolepis A. Gray an octoploid with 72 members in its complement (fig. 15, 16). The chromosomes of S. cryptandrus are rod-like to U-shaped in form, usually about  $0.7~\mu$  in diameter and  $2.5~\mu$  to  $6.0~\mu$  in length. Well-defined subterminal and submedial constrictions and a pair of satellites were observed. The nuclear diameters averaged  $7.8~\mu$ . The chromosomes of the S. heterolepis material studied were similar in form to those of S. cryptandrus but showed no morphological peculiarities. Their average diameter was  $0.5~\mu$ , and the lengths varied from  $1.0~\mu$  to  $3.9~\mu$ . The nuclear diameters averaged  $10.5~\mu$ .

Stipa pulchra Hitch. and S. columbiana Macoun, respectively, are hexaploid with 66 chromosomes and tetraploid with 44 chromosomes (fig. 17, 18). In S. pulchra the chromosomes are slightly less than 0.5  $\mu$  in diameter and from 1.0  $\mu$  to 3.0  $\mu$  in length. Two pairs of the usually rod-like to U-shaped chromosomes have deep subterminal constrictions. The average nuclear diameter was calculated as 10.0  $\mu$ . The chromosomes of S. columbiana are very similar to those of S. pulchra but average slightly over 0.5  $\mu$  by 1.5  $\mu$ . No significant variations in their morphology were observed. The diameter of the nuclei of

S. columbiana averaged 7.6 μ.

That there are exceptions to these basic numbers of 7, 9, and 11 found in the species of the tribe Agrostideae has been indicated by other workers. Thus Avdulov (1928, 1931) reported diploid numbers of "about" 40 chromosomes in Muhlenbergia glomerata (Willd.) Trin. (M. racemosa (Michx.) B. S. P.), of 40 in M. mexicana (L.) Trin. and in M. umbrosa Scribn. (M. sylvatica Torn.). Humphrey (1937) and Nielsen and Humphrey (1937) reported finding 42 chromosomes in somatic tissue of Muhlenbergia pungens Thurb. However, the present writer has rechecked material of this spe-

cies and found it to have 60 chromosomes (fig. 19). The members of the complement are irregular in form, varying from 1.0  $\mu$  to 3.5  $\mu$  in length, and are approximately 0.3  $\mu$  in diameter. Several pairs have medial or subterminal constrictions, and one pair of satellites was observed. Further, Avdulov (1928) reported complements of 24 in Oryzopsis miliacea (L.) Benth. and Hook., 24 in O. virescens (Trin.) Beck, and 38 in Urachne trichotoma (Nees) Trin.

Chlorideae.—Avdulov (1931) reported the somatic chromosome number of Buchloë dactyloides (Nutt.) Englem. to be 60. All the figures studied by this writer have 56 short and predominantly straight chromosomes mostly less than  $0.5 \mu$  in diameter and  $0.7 \mu$  to  $2.0 \mu$  in length (fig. 20). The

nuclear diameters average 10.1 μ.

Other instances somewhat comparable to that indicated in Buchloë occur in other genera and species of this tribe. Complements of 56 and 60 have been independently observed and reported to occur within species of Bouteloua and Spartina. Avdulov (1931) observed 40 chromosomes in Bouteloua gracilis (H. B. K.) Lag. Humphrey (1937) and Nielsen and Humphrey (1937) reported that Americangrown material of the same species had 42 chromosomes. Fults (1938), in personal correspondence to the writer, stated, "First, in regard to blue grama-I have found complements ranging all the way from 14 to apparently about 77, some of them being aneuploids and some of them being euploids. I have some meiotic figures showing the association and distribution of chromosomes in the first meiotic divisions and have found spindles with apparently all manner of types of association and distribution." In the genus Spartina, Avdulov (1928) observed complements of 40 in S. Schreberi F. Gmel., and of 80 to 90 in S. cynosuroides (L.) Roth. Huskins (1930) observed 70 chromosomes in S. alternifolia Lois, 56 in S. stricta (Ait.) Roth, and 126 in S. Townsendii H. and J. Groves. In 1929 Church reported finding 14 bivalents in S. pectinata Link (S. Michauxiana Hitch.) and 14 bivalents and 14 univalents in S. alternifolia var. glabra (Muhl.) Trin. Some species of the genus Chloris also have complements occurring in multiples of 7 and 10. Avdulov (1928) reported finding 20 chromosomes in C. barbata (L.) Swartz (C. inflata Link) and C. Gayana Kunth and 40 in C. acuminata Trin. (C. distichophylla Lag.), C. cucullata Lag., and C. truncata R. Br. In 1931

Fig. 1-33. Chromosome complements in somatic tissue.\*—Fig. 1. Bromus inermis ( $\times 2600.\ 2n=56$ ).—Fig. 2. B. inermis ( $\times 2100.\ 2n=70$ ).—Fig. 3. B. marginatus ( $\times 2600.\ 2n=70$ ).—Fig. 4. B. commutatus ( $\times 2100.\ 2n=56$ ).—Fig. 5. B. secalinus ( $\times 2100.\ 2n=14$ ).—Fig. 6. Glyceria grandis ( $\times 2100.\ 2n=28$ ).—Fig. 7. Eragrostis curvula ( $\times 2600.\ 2n=40$ ).—Fig. 8. E. sessilispica ( $\times 2600.\ 2n=40$ ).—Fig. 9. E. spectabilis ( $\times 2600.\ 2n=40$ ).—Fig. 10. Elymus canadensis var. robustus ( $\times 1500.\ 2n=28$ ).—Fig. 11. E. Macounii ( $\times 2600.\ 2n=28$ ).—Fig. 12. E. sibericus ( $\times 2600.\ 2n=28$ ).—Fig. 13. Calamagrostis epigeios ( $\times 2100.\ 2n=28$ ).—Fig. 14. Agrostis exarata ( $\times 2600.\ 2n=28$ ).—Fig. 15. Sporobolus cryptandrus ( $\times 2600.\ 2n=18$ ).—Fig. 16. S. heterolepis ( $\times 2600.\ 2n=72$ ).—Fig. 17. Stipa pulchra ( $\times 2600.\ 2n=66$ ).—Fig. 18. S. columbiana ( $\times 2600.\ 2n=44$ ).—Fig. 19. Muhlenbergia pungens ( $\times 2600.\ 2n=42$ ).—Fig. 20. Buchloë dactyloides ( $\times 2600.\ 2n=60$ ).—Fig. 21, 22. Paspalum Urvillei ( $\times 2600.\ 2n=40$ ) and 60, respectively).—Fig. 23. Panicum anceps ( $\times 2600.\ 2n=18$ ).—Fig. 24. P. Lindheimeri ( $\times 2100.\ 2n=18$ ).—Fig. 25. P. tevanum ( $\times 2600.\ 2n=36$ ).—Fig. 26. Andropogon furcatus ( $\times 2600.\ 2n=60$ ).—Fig. 27, 28. A. Hallii ( $\times 2600.\ 2n=60$ ) and 70, respectively).—Fig. 29, 30. A. scoparius ( $\times 2600.\ 2n=40$ ).—Fig. 31, 32. A. saccharoides ( $\times 2600.\ 2n=60$ ) and 70, respectively).—Fig. 33. A. perforatus ( $\times 2600.\ 2n=80$ ).

<sup>\*</sup> In reproducing this plate the lineal dimensions have been reduced approximately one-half.

he reported observing 80 chromosomes in C. submutica H. B. K., 40 in Trichloris mendocina (Phil.) Kurtz. The same year he also reported 18 chromosomes in Eleusine indica (L.) Gaertn., 36 in E. corocana (L.) Gaertn., and 14 in Beckmannia eruciformis Hook. Rau (1929) had previously reported the "probable" diploid number in the root tips of E. corocana to be 36. Krishnaswami and Ayyangar (1935) confirmed these reports for E. indica and E. corocana and reported haploid numbers of 18 in E. brevifolia R. Br. and 17 in E. aegyptica Desf. Nielsen and Humphrey (1937) observed 14 chromosomes in Chloris virgata Swartz and in Beckmannia syzigachne (Steud.) Fern.

Paniceae.—Two cytogenetic races of Paspalum Urvillei Steud., possessing complements of 40 and 60 chromosomes, respectively, were investigated (fig. 21, 22). The chromosomes of the former race were slightly larger than those of the latter, otherwise no visible difference between the members of the complements was observed. The nuclei of the latter race were found to be the larger. A comparison of the plants growing under field conditions shows that the race with the larger chromosome number is characterized also by more robust plants. In addition to the coarser culms and slightly longer racemes, the plants of the 60-chromosome race were pubescent on the sheaths, particularly along their margins, whereas those of the 40-chromosome race were glabrous excepting for a slight trace of pubescence upon the very lowermost sheaths.

Three species of Panicum were investigated. Of these P. anceps Michx. and P. Lindheimeri Scribn. are diploid species each having 18 somatic chromosomes, while P. texanum Buckl. is tetraploid (fig. 23, 24, 25). The individual chromosomes are morphologically similar, being for the most part either rod-shaped or somewhat crescent-shaped in form. Those of P. anceps were slightly less than  $0.5 \mu$  in diameter and  $1.5 \mu$  to  $3.5 \mu$  in length. The nuclei average  $5.0 \mu$ . P. Lindheimeri is intermediate, with chromosomes  $0.5 \mu$  in diameter,  $1.5 \mu$  to  $2.5 \mu$  long, and nuclei  $7.2 \mu$  in diameter. P. texanum has the largest chromosomes of the three species. They average  $0.7 \mu$  by  $1.5 \mu$  to  $5.0 \mu$ , and its nuclei are  $9.5 \mu$  in diameter.

These species of Panicum and Paspalum fall into two series with basic numbers of 9 and 10, respectively. In regard to other members of this tribe Karper and Chisholm (1936) reported observing 14 chromosomes in Pennisetum glaucum (L.) R. Br. Likewise, Church (1929) reported 42 chromosomes in Echinochloa crusgalli (L.) Beauv. and 56 in its variety frumentacea W. F. Wright. However, Avdulov (1931) reported observing 54, and Hunter (1934) found 36 chromosomes in what is presumably this same variety of Echinochloa.

Andropogoneae.—In 1929 Church reported finding 60 chromosomes in Andropogon furcatus Muhl. (A. provincialis Lam.). The writer has confirmed this observation (fig. 26). The figures examined showed very little difference among the in-

dividual members of the complement other than variations from short rod-like to strongly U-shaped bodies. They are prevailingly about 0.7  $\mu$  in diameter and vary from 2.0  $\mu$  to 4.0  $\mu$  in length. The nuclei average 9.0  $\mu$  in diameter.

Andropogon Hallii Hack., as it appears in the field, is exceedingly polymorphic, some races appearing to blend gradually with certain races of A. furcatus. Two of the six distinctly different morphological types selected from a single blowout in the sandhills near Inez, Nebraska, yielded material suitable for cytological study. Complements of 60 and 70 chromosomes were observed (fig. 27, 28) in selections No. 35 and No. 33, respectively. As is indicated in table 1, the individual chromosomes are nearly alike both in diameter and length. The nuclear diameters in the 70-chromosome race are slightly larger than in the 60-chromosome race. A comparison of fruiting plants of these selections indicates the following morphological differences distinguish the two races:

## No. 33

Lowermost leaf of fruiting culm 0.7 to 0.8 cm. wide, glabrous.

Pedicels of sterile spikelets usually less than 5 mm. long, pubescence sparse, white on growing plants.

Caryopsis mostly over 5.5 mm. long, slender.

Sterile spikelet awns 5 to 7 mm. long.

# No. 35

Lowermost leaf of fruiting culm 1.0 to 1.1 cm. wide, densely pilose.

Pedicels of sterile spikelets mostly more than 5 mm. long, pubescence dense, dark yellow on growing plants.

Caryopsis mostly less than 5 mm. long, plump.

Sterile spikelet awnless.

Two races of Andropogon scoparius Michx. were found to be characterized by complements of 40 chromosomes, which differ somewhat in structure. In one race, No. 856, no striking morphologic irregularities in chromosome structure were found, while the second race, No. 848, had one pair of deep subterminally to submedially constricted chromosomes. An examination of the other data given in table 1 and figures 29 and 30 indicates a well-defined difference in chromosome size and a small difference in the nuclear diameters. Growing plants and herbarium material taken from these selections show the following morphological differences characterize the two races:

## No. 848

Pilose lower sheaths.

Foliage dense and leafy.

Inflorescence loose and more erect.

Plant somewhat glaucous. Spikelet pubescence about 3 mm. long.

No. 856

Glabrous lower sheaths. Plant with sparse foliage. Inflorescence dense and spreading.

Plant not glaucous.

Spikelet pubescence about 2.7 mm. long.

A situation closely paralleling the case just reported was found to exist in *Andropogon saccharoides* Swartz, of which two cytogenetic races, characterized by 60 and 70 chromosomes, respectively,

were studied (fig. 31, 32). The data given in table 1 show that the 60-chromosome race, No. 860, has slightly thicker chromosomes, which vary from 1.0  $\mu$ to 4.0 \(\mu\) in length, as compared with the slightly thinner members of the 70-chromosome race, No. 859, which range from 1.5  $\mu$  to 3.5  $\mu$  in length. In 1931 Avdulov reported the diploid chromosome number of this species as 60. An examination of herbarium material taken from these plants showed the following differences:

#### No. 859

Plant with coarse culms, abundant foliage.

Lower sheaths pilose.

Awns mostly about 2 cm.

Terminal racemes about 4 cm. long.

# No. 860

Plant with fine culms, sparse foliage.

Lower sheaths glabrous.

Awns mostly about 1.4 cm. long.

Terminal racemes about 3.5 cm. long.

It may also be significant that No. 860 was heavily infected with Tolyposporium bullatum Schr., whereas No. 859, which was grown on the adjoining plot in the nursery, was free from the fungus.

Root-tip material of Andropogon perforatus Trin. showed complements of 80 chromosomes (fig. 33). Their form varies from rod-like to U-shaped in outline. They average  $0.5 \mu$  in diameter and range from 1.5  $\mu$  to 5.0  $\mu$  in length. The average diameter of the nuclei is 9.7  $\mu$ .

In this tribe all the observed chromosome complements were in multiples of 10. That species with complements not in multiples of 10 occur in the Andropogoneae is indicated by Bremer's (1934) report of finding 38 chromosomes in Miscanthus japonicus (Thumb.) Anderss., by Church's (1929)

report of 42 chromosomes in M. sinensis Anderss. and by Hunter's (1934) observation of 64 chromosomes in M. saccharifer (Anderss.) Benth. Other deviations from the basic number 10 have been reported by Avdulov (1931), who found 54 chromosomes in Rottboellia glandulosa Trin. (Manisuris glandulosa Trin.), and by Kuwada (1919) who reported observing 68 chromosomes in Ischaemum anthephoroides Miq.

#### SUMMARY

The diploid chromosome numbers in 34 races belonging to 28 species of the Gramineae are reported. The basic numbers occurring in the species studied are indicated, and attention is called to a few species with complements in multiples of numbers other than those common in the particular tribe concerned. In the study of species of the Chlorideae, several species are indicated wherein chromosome complements in multiples of both 7 and 10 have been reported.

Two new races, both characterized by 70 chromosomes, were observed in Bromus marginatus and in B. inermis, and one race with a diploid complement of 14 was found in B. secalinus. These complements appear to be new for the polyploid series occurring

in those species.

Complements of 40 and 60 somatic chromosomes were observed in races of Paspalum Urvillei, and others of 60 and 70 chromosomes were observed in both Andropogon Hallii and A. saccharoides. The vegetative features of these races are compared.

The findings are presented in table 1.

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# STRUCTURE AND GROWTH OF THE SHOOT APEX OF CYCAS REVOLUTA $^{1}$

Adriance S. Foster

RECENT STUDIES on the shoot apex of vascular plants emphasize our inadequate knowledge of the fundamental nature of growth in terminal meristems. In particular, the investigations of Korody (1937), Härtl (1938), and Foster (1938) have clearly shown the complex interrelationship between the structural pattern and the distribution of growth in the meristem as a whole and its bearing on organ and tissue differentiation in the shoot. It is all too evident, however, that the classical concept of "primordial meristem" needs serious re-examination along broad comparative lines. For example, such phenomena as mitosis, cytokinesis, vacuolation, and cell enlargement are obviously concomitant with growth and differentiation, but their interrelationships in apical meristems at present are obscure and extremely complex.

The shoot apex of gymnosperms affords unusually favorable material for studies in this field because of the marked segregation in many genera of cell zones distinguished by such features as reaction to stains, frequency of mitosis, planes of division, and relative thickness of walls. In the present article, the results of a study on the shoot apex of several cycads are described. Since cycads possess a number of unique and primitive morphological characters, a preliminary exploration of their apical meristems should produce data of considerable interest when viewed comparatively with the situation in conifers (Korody, 1937), Ginkgo (Foster, 1938), and the angiosperms (cf. references in Foster 1936, 1938). The major emphasis in this study has been given to the shoot apex of seedlings and adventitious buds of Cycas revoluta Thunb. A less intensive examination has been made of the shoot tips of embryos, seedlings, and adult plants of Zamia floridana A.DC.

Doubtless the great difficulty of securing an abundance of critical material is responsible for the meagre literature on the structure of the shoot apex in cycads. The earliest account was given by Hofmeister (1857, taf. VII, fig. 12), who depicted a surface view of the shoot tip of Zamia longifolia to illustrate his belief in the existence of a triangular apical cell. No description is furnished, however, and his figure does not support his own interpretative received for publication April 4, 1939.

tion. A more detailed account of the presumptive apical cell in the seedling apex of Ceratozamia sp. was given by Dingler (1882, p. 30-33; taf. I, fig. 1-4), but the entire structure of the meristem is not described or figured. In contrast to Hofmeister and Dingler, Strasburger (1872, p. 335-336; taf. XXV, fig. 35-36) found no evidence of a single apical cell in the apex of adventitious buds of Cycas revoluta. He concluded that new cells originate from two superposed tiers of initials, the upper producing the dermatogen-periblem region, the lower the "massive plerome." Strasburger's general viewpoint was supported by Warming (1877, p. 119) for Ceratozamia longifolia and Cycas circinalis, by Bower (1884, p. 584) for Cycas Seemanni, and was adopted by DeBary (1884, p. 14) in his compendium on anat-

Within recent years, many studies have been made on the embryo, seedling, and adult shoot of various cycads without, however, appreciably advancing our knowledge of the growth and structure of the shoot apex (cf. bibliographies in Schuster [1932] and Chamberlain [1935]). Indeed, as far as the writer is aware, Thiessen (1908, p. 363; pl. XXV, fig. 8) is the only investigator who has attempted a description of the entire cellular structure of the apex. Working on the embryo of Dioon edule he concluded that the procambium, which is "the most active meristematic region of the stem," arises very near the growing point and that the pith "terminates in a tier of cells against the epidermis." Chamberlain (1910) emphasizes the "late differentiation" of dermatogen, periblem, and plerome regions in the shoot tip of the embryo of Dioon edule but gives no information on the structure of the terminal meristem of the seedling or adult plant of this species. Other workers (Dorety, 1908, 1909, 1919; Matte, 1909) entirely neglect the organization of the apical meristem.

MATERIALS AND METHODS.—The materials used in this investigation were furnished by the following individuals to whom grateful acknowledgment is made: Mr. Robert MacMillan of the City Nursery of Pasadena, California, for about five dozen two-year old seedlings of Cycas revoluta; Mr. Louis B. Downs, head gardener at Central Park, Pasadena, for his assistance in obtaining numerous adventi-

tious buds of *Cycas revoluta*; Dr. W. S. Phillips of Miami University, Coral Gables, Florida, for seeds and many fine adult plants of *Zamia floridana*; and Professor C. J. Chamberlain, University of Chicago, for several seedlings of *Zamia floridana*.

The same methods of fixation, staining, and illustration employed in my study of Ginkgo (Foster, 1938) were adopted. Serial transverse and longitudinal series were cut at 6 and 8 microns, the former thickness proving more satisfactory. Great difficulty was experienced in securing perfect series through the apex of adventitious buds of Cycas revoluta, even after removing the majority of the cataphylls. The use of a water-cooling device for knife- and block-carrier (cf. Chamberlain, 1932, p. 113) was found to be essential. I am indebted to one of my students, Mr. Ernest Ball, for planning the construction of this attachment.

Thanks are due my wife for her usual invaluable assistance with the illustrations. I am further indebted to the Board of Research of the University of California for a grant during the investigation.

GENERAL MORPHOLOGY OF THE BUDS OF CYCAS REVOLUTA.—In the two-year-old seedlings used for this study, the two cotyledons were still attached to the gametophyte of the seed, and the prominent conical terminal bud consisted of an outer series of tough cataphylls, one or two expanding or mature pinnate foliage leaves, and a highly variable number of developing foliar primordia. The adult cataphyll in C. revoluta consists of a massive sheath bearing a conspicuous median pointlet which morphologically represents an undeveloped lamina. In the dissection of about 40 buds for histological study it was observed that the early stages in ontogeny of cataphyll and foliage leaf appeared remarkably similar, at least externally, the principal distinction being the early appearance in the foliage leaf of the initials of the lateral pinnae. Utilizing this criterion, the foliar structures surrounding the shoot apex in the illustrations in this paper are regarded as cataphyll primordia. The general structure of the adventitious buds developing on the trunks of C. revoluta is similar except that the cataphylls are larger and more numerous and the foliage leaves tend to be arranged in definite "crowns." According to Stopes' (1910) observations on cultivated specimens of C. revoluta growing in Japan, the adventitious buds arise from the inner living tissue of the persistent leaf-bases of the trunk and are not connected in any way with the axis at first. A histogenetic study of the origin and early development of such buds would be of considerable interest. Stopes concludes that "the behavior of the buds makes it clear that probably the great majority of 'branched' plants are simply those in which one or more of these adventitious 'buds,' developed from the leaf-bases, had grown till it rivalled the main axis."

When viewed in median longisection, the shoot apex of seedlings typically has the form of a promi-

nent asymmetrical cone which is almost completely overarched by the adjacent cataphyll primordia (pl. I, fig. 1). In these respects, it contrasts markedly with the slightly convex and less extended shoot apex of Ginkgo (Foster, 1938, p. 533, fig. 1-2). The apex of adventitious buds of C. revoluta is much larger than that of the seedling and appears as a rather symmetrical blunt cone which, as was emphasized by Strasburger (1872), is depressed between and overarched by the adjacent leaf primordia (pl. I, fig. 6).

ZONAL STRUCTURE OF THE SHOOT APEX - The embryonic tissue of the shoot apex of seedlings of C. revoluta is not uniform in its structure or reaction to stains but on the contrary exhibits a characteristic zonation. In median longisection it is evident that a prominent core of relatively large, irregularly-arranged lightly-stained cells extends through the center of the apex, terminating as a distinct zone near or at the surface of the latter (pl. I, fig. 1, 2, 5). This internal core, which will be designated as the central tissue, is completely surrounded by a zone of peripheral tissue, composed of somewhat smaller cells with deeply-stained protoplasts (pl. I, fig. 1-5). An examination of numerous longisections demonstrates that these two well-defined tissuezones do not arise from separate tiers of initials. On the contrary, they originate from a single terminal group of initial cells which divide, apparently in no fixed sequence, both anticlinally and periclinally.

Figure 1 represents schematically the writer's interpretation of the distribution of growth in the peripheral and central tissue-zones of the apex. This diagram is based upon a detailed study of the orientation of mitotic figures, the relative frequency of new periclinal and anticlinal walls, and the degree of cell enlargement, in both longitudinal and transverse sections of many apices. Growth in the core of central tissue (zone II) is predominantly anticlinal with respect to the surface of the apex and is associated both with the division and highly irregular enlargement of the cells (pl. I, fig. 2-5). In contrast, the peripheral tissue (zone III) constitutes a zone typified by marked centrifugal growth in thickness chiefly resulting from repeated periclinal and anticlinal divisions in the surface cells and their derivatives on the flanks of the apex. In figure 1, the demarcation between these two zones is shown by the broken lines. It must be emphasized at this point, however, that such a demarcation is to some extent arbitrary, since many preparations conclusively show how irregular groups of cells, at the inner boundary of the peripheral tissue, may originate from the central tissue (fig. 4d-f, 6d, 7e). Under such circumstances, the relative depth of the peripheral tissue may be expected to vary somewhat in various apices, apparently in relation to fluctuations in the degree of radial expansion of the central tissue at a given level.

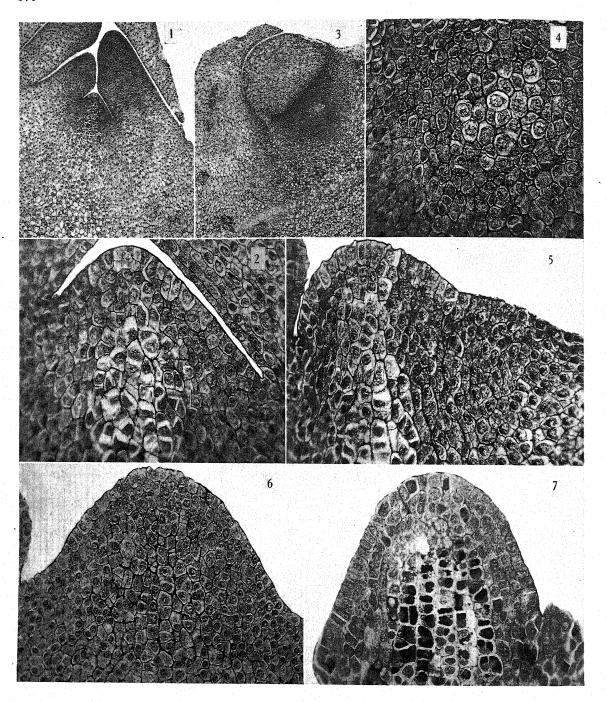


Plate I, fig. 1-7.—Fig. 1. Approximately median longisection of a seedling bud of Cycas revoluta showing the asymmetrical shoot apex overarched by the two youngest cataphyll primordia. The deeply-stained peripheral tissue of the shoot apex contrasts markedly with the prominent core of central tissue from the base of which the broad pith originates. Small granular bodies in the pith cells are starch grains. The procambial tissue, at each side of the pith, differentiates from the inner and lower portion of the peripheral tissue. Note the procambial tissue in the lower portion of the cataphyll primordium at the right and the two leaf-trace "girdles" in the cortex at the lower right of the figure.  $\times 36.5$ .—Fig. 2. The shoot apex of fig. 1 at higher magnification ( $\times 217.5$ ), illustrating the differences in size, arrangement, and staining reactions of the cells of central and peripheral tissue. Note the thickened areas of walls particularly in the central tissue and the active centrifugal growth in thickness of the peripheral tissue. This section is adjacent to the one shown in text fig. 6d.—Fig. 3. Transection of seedling bud of Cycas revoluta, 176 microns from surface of shoot apex, illustrating the clearly defined central tissue (small central group of lightly-stained cells) completely surrounded by the deeply-stained peripheral tissue. Note the leaf-trace "girdles" in the cortex at

With this general description of the distribution of growth in the shoot apex as a basis, a detailed account of the cellular structure of the meristem can now be given.

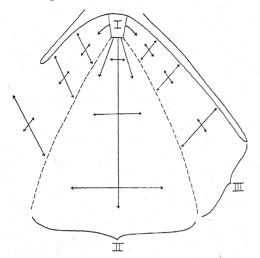


Fig. 1. Diagram illustrating the zonal structure and the distribution of growth in the shoot apex of seedlings of Cycas revoluta. Zone I is the apical initial group from the cells of which by anticlinal and periclinal divisions the peripheral tissue (zone III) and the core of central tissue (zone II) originate. The approximate cytological demarcation between zones II and III is represented by the broken outlines. In zone III, active centrifugal growth in thickness (long double arrows perpendicular to the surface) dominates over the less conspicuous growth in surface (smaller double arrows). The growth in zone II is predominantly anticlinal with respect to the surface of the shoot apex (long single arrow) but is obscured by progressive centrifugal expansion, as illustrated by the increasing length of the three double arrows.

APICAL INITIAL GROUP (zone I, fig. 1).—Although it is conceivable that an apical cell may be present at some phase in the embryogeny of *C. revoluta*, as is the case in certain of the Abietineae (Buchholz, 1920), no evidence of the permanent existence of such a cell appears from my investigations on the apices of seedlings and adventitious buds. It is true that certain preparations or individual sections in a series might seem to indicate the existence of a definitive apical cell. For example, in the surface

view of a seedling apex shown in figure 4a, a small triangular cell, with a recent division wall parallel to one of its sides, is apparent. This cell, also shown 8 microns below the surface in figure 4b, might easily be interpreted as a typical apical cell, and its immediate neighboring cells as "segments." Indeed, the cell-net in figure 4a bears a striking resemblance to Dingler's (1882, taf. I, fig. 1) figure of the presumptive apical cell and its recent segments in the shoot apex of a seedling of Ceratozamia. However, surface views of several other seedling apices in C. revoluta showed a highly irregular arrangement of cells and clearly suggest that the cell net depicted in figures 4a-b merely represents a transitory "constellation" in an ever-changing cell pattern. This conclusion is firmly supported by a careful examination of thin serial longisections through about 40 shoot apices. Sections cut in this plane show that no fixed or regular scheme of segmentation characterizes the terminal cells in the median region of the apex. Occasionally, a cell may be found in a median longisection which suggests in its form a definitive apical cell (fig. 8). However, there is no evidence that such a cell is "permanent" in character or regular in plane of cleavage. Furthermore, serial sections clearly demonstrate that essentially similar wedge-shaped cells may arise distal to the median plane by the oblique and unequal division of surface cells (fig. 2a-b, 5a-b, 9a, 11). More frequently, the geometrical apex of the terminal meristem is occupied by a relatively large prismatic cell, around which are grouped its most recent derivatives. In some preparations, the lateral derivatives of this prismatic "initial" have divided periclinally, thus producing a comparatively regular group of related cells (pl. I, fig. 2; fig. 3b-c, 5b. 6b-d). In other apices, no such regular segmentation obtains, and it is evident that the sequence of anticlinal, periclinal, and oblique divisions in the presumptive initial and its derivatives fluctuates within wide limits (pl. I, fig. 5, 6; fig. 2a-b, 7e-f, 10a-b). A similar condition is apparently also characteristic of the seedling apex of Zamia floridana, although no intensive study of this species has been made (fig. 13).

In view of the wide variations just described in respect to the shape and planes of division of the terminal cells in the shoot apex, it is impossible to

the extreme left of the figure.  $\times 36.5$ .—Fig. 4. The central and peripheral tissue zones of fig. 3 at higher magnification ( $\times 217.5$ ). Note the thick walls, large nuclei and collapsed lightly-stained cytoplasm of the large central cells. For emphasis and clarity, this section is also shown as text fig. 4g.—Fig. 5. Longisection through shoot apex of a seedling of C. revoluta to illustrate contrast between central and peripheral tissue and the method of cataphyll initiation (right flank). Observe the notable radial depth of the cells of the apical initial group, one of which has experienced a typical T-division. This section is 16 microns distal to text fig. 2a.  $\times 217.5$ .—Fig. 6. Approximately median section of the shoot apex of an adventitious bud from the trunk of a large specimen of C. revoluta. This massive cone (maximum width 460 microns, maximum height 276 microns) illustrates the structure typical of slowly-growing shoots. Note that the central tissue is well defined and consists of prominent but irregular cell groups, the "mother" walls of which are irregularly thickened especially at the corners. A metaphase is visible in a central cell near the base of the figure. Periclines are evident in many of the surface cells of this apex.  $\times 150$ .—Fig. 7. Approximately median longisection of the shoot apex of Abies venusta. The peripheral tissue (note telophase at upper right) consists of several "layers" of deeply-stained cells which surround the prominent core of central tissue. The darkly-stained material in the central cells presumably represents tannin. Contrast the comparatively orderly arrangement of the cells in the peripheral and central tissue with their "homologues" in Cycas (fig. 2, 5, 6).  $\times 217.5$ .

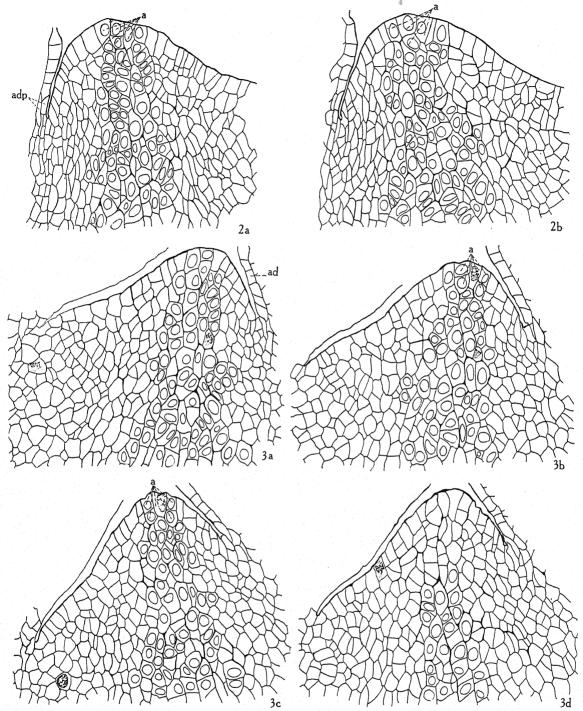


Fig. 2-3. Longisections of shoot apices of seedlings of *C. revoluta*. In these and all following figures, the extent of the central tissue (zone II, fig. 1) is indicated by nucleated cells, while the cells of the peripheral tissue (zone III, fig. 1) are shown in outline only. In the presumptive median section or sections, nuclei are also represented in the cells and immediate derivatives of the apical initial group (zone I, fig. 1). Localized wall-thickenings in all regions are demarcated by heavy outlines, surface views of primary pit-fields by stippling. Fig. 2a-b, adjacent sections through median region of apex illustrating origin of central and peripheral tissue from the apical initial group. Note especially the two large cells in the central tissue in fig. 2b. Fig. 5, pl. I represents a section from the same apex 16 microns distal to fig. 2a. Fig. 3a-c, adjacent sections through median region of another apex, showing an unusually regular segmentation of the cells of the apical initial group; fig. 3d 12 microns distal to fig. 3c. Legend: a, cells of apical initial group; ad, adaxial surface of cataphyll primordium; adp, periclinal division of adaxial surface cells of cataphyll primordium. All figures ×217.5.

determine the theoretical number of "permanent" initial cells. In my opinion, the evidence points to the conclusion that a varying number of fundamentally similar cells act in unison as an apical initial

group from which arise the peripheral and central tissue-zones of the apex. It also seems justifiable to conclude that the growth and division of cells in the apical initial group are not primary and "deter-

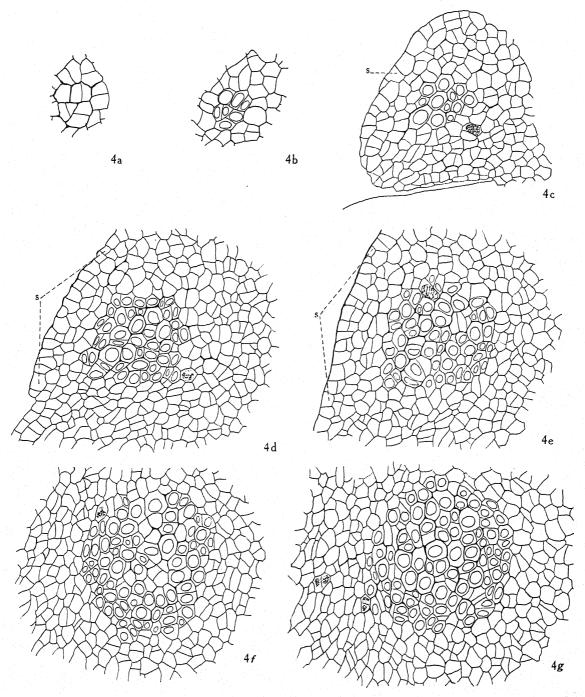


Fig. 4a-g. Transections through shoot apex of seedling of C. revoluta.—Fig. 4a. Surface view of cells of apical initial group.—Fig. 4b-4g, sections at levels respectively of 8, 56, 80, 112, 160, and 176 microns below fig. 4a. Note that while the majority of cells in the central tissue are highly variable in size and arrangement, a marked tendency to radial alignment is shown at the margins of this zone in relation to the propagation of the inner portion of the peripheral tissue. The marked contrast between central and peripheral tissue is shown for fig. 4g in pl. I, fig. 4. Legend: a, apical initial group; s, surface cells of flank of shoot apex.  $\times 217.5$ .

minative" processes but instead are subordinated to the general distribution of growth in the shoot apex as a whole.

THE CENTRAL TISSUE (zone II, fig. 1).—The zone of central tissue has the form of a broad-based cone

which terminates in the uppermost median region of the shoot apex. Since precise orientation of bud material on the block carrier of the microtome is virtually impossible, many series were obtained in this investigation which were obviously oblique to

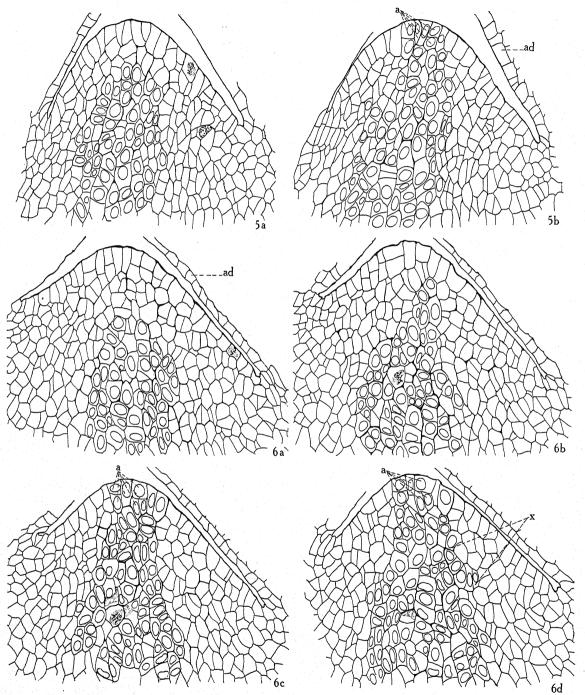


Fig. 5-6.—Fig. 5a-b, longisections through shoot apices of seedlings of *C. revoluta*; fig. 5b, approximately median and 30 microns distal to fig. 5a. Fig. 6a-d, longisections through another apex. Fig. 6c-d, median; fig. 6a-b, respectively 36 and 12 microns distal to fig. 6d. Note surface views of primary pit-fields in fig. 6c-d and origin of group of peripheral cells from edge of central tissue in fig. 6d at x. Fig. 2, pl. I represents the section following fig. 6d in the same apex. Legend: a, apical initial group; ad, adaxial surface of cataphyll primordium. All figures ×217.5.

the median axis of the apex. Consequently, the sections used for illustrations in this paper were selected only after a careful examination of a wide series of preparations. The determination of the "median" longitudinal section or sections was based upon (1) the symmetry and contour of the sections at either side and (2) the relative length and breadth of the zone of central tissue. These criteria will become evident from an examination of figures 6 and 7, which show the change in form and proportion of the central tissue at progressive levels in two shoot tips. The conical form of the zone of central tissue is likewise clearly illustrated by the series of transverse sections shown in figure 4.

The central tissue of the shoot apex of both seedlings and adventitious buds arises from a group of subterminal "mother cells" which represent the inner derivatives of the cells of the apical initial group (pl. I, fig. 2, 5, 6; fig. 2a-b, 3a-c, 5b, 6c-d, 10a-b). There apparently is no orderly plan of growth or division in these mother cells. In some apices, repeated periclinal divisions (with respect to the geometrical apex) result in short rather welldefined groups or files of cells which can be clearly traced to the apical initial group (fig. 2a, 3a-b, 7e-f, 10a). Other preparations, however, show an exceedingly variable pattern resulting from the enlargement and division in many planes of the mother cells (pl. I, fig. 2, 5; fig. 5b, 6c-d). This is particularly true of the apex of adventitious buds where the subterminal mother cells as seen in both transverse and longitudinal section are arranged in complex cell-groups suggestive of Schüepp's (1926, p. 16-17) "Massiges Meristem" (pl. I, fig. 6).

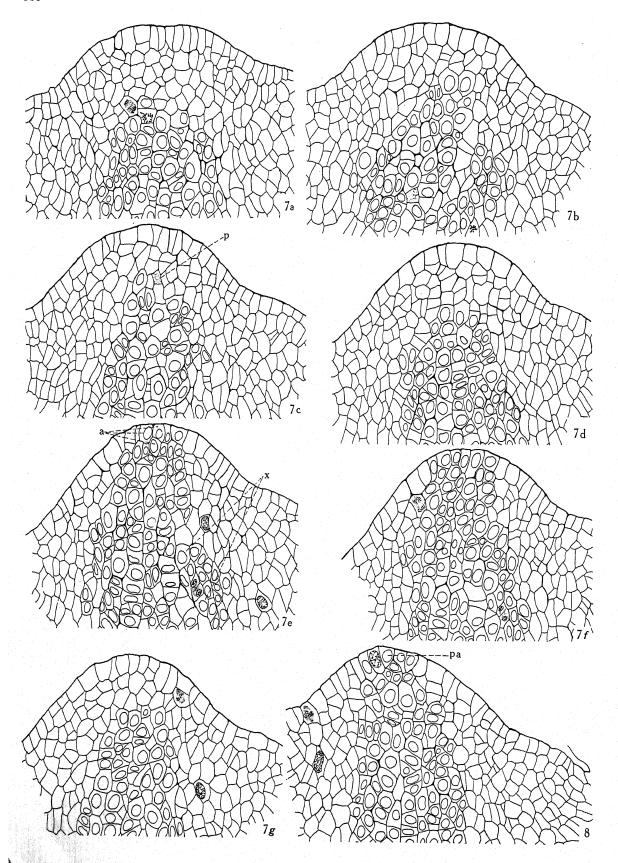
The gradual basipital differentiation of the central tissue is marked by the relatively enormous increase in size of many cells and the subsequent formation from them of irregular cell groups. Unquestionably this is one of the fundamental processes characteristic of the central tissue and is responsible for the highly variable size and arrangement of cells which distinguishes this zone. Although no statistical study of cell size was made, a few representative measurements indicative of the order of magnitude may be of interest. For example, the dimensions (i.e., length × width in microns) of the two elongated upper and lower central cells at the upper left of figure 2b are respectively 44 imes 16.5 and 40.7 imes 13.2. Other prominently enlarged central cells, more nearly "isodiametric" in form, vary from 25 to 35 microns in maximum length and width. The volume of such cells cannot be accurately determined but clearly approaches the order of 7000-8000 cubic microns.

As stated above, the enlargement of a given central cell is eventually followed by a series of divisions, resulting in the production of a genetically-related cell group. If the direction of enlargement is more or less strictly vertical with respect to the longitudinal axis of the shoot apex, filament-like series of cells are produced which collectively re-

semble a typical rib meristem (Schüepp, 1926). This type of growth and cell formation is particularly obvious in the lower region of the central tissue but may occur, in varying degrees, at any level in the zone (pl. I, fig. 2, 5; fig. 2, 3, 5-10, 12). It clearly reflects the notable emphasis on anticlinal growth typical of the central tissue zone (cf. fig. 1). At any point in the central tissue, however, cells may expand more or less uniformly in all directions. The sequence of anticlinal, periclinal and oblique divisions in such cells is highly variable, and the exact origin of the resulting cell-groups is hence difficult to determine (cf. especially fig. 3a-b, 4d-f, 6b-d, 10a-b). This type of cell behavior indicates that irregular radial expansion accompanies the vertical extension of the cone of central tissue (fig. 1). Additional evidence of this complex interrelationship is shown by the frequent radial enlargement of cells at the edge of the central tissue and the production of cell groups which become incorporated in the inner portions of the peripheral tissue (pl. I, fig. 2, 4, 5; fig. 6d, 7e, cell-groups labelled X). Under such circumstances, it should be apparent that the "limits" between the central and peripheral tissue zones can be only approximately established in the shoot apex of seedlings. These zones clearly have no rigid histological boundaries but on the contrary are mutually interdependent. A similar relationship is likewise shown in the massive apices of adventitious buds (pl. I, fig. 6).

Aside from the mode of growth and cell formation just described, several additional characteristics help to demarcate the cells of the central tissue. One of the most obvious of these features is the conspicuous plasmolysis of many of the protoplasts as contrasted with the more satisfactory fixation of the cells of the adjacent peripheral tissue (pl. I, fig. 1-5). It seems reasonable to conclude that the marked collapse of the lightly-stained cytoplasm in the central cells is correlated with their highlyvacuolated state during the phase of enlargement. In order to test this assumption, a large number of seedlings are now being grown so that the effects of a wide range of killing and fixation reagents may be determined. It is also hoped that observations on living meristem, with the aid of vital stains, may prove feasible.

Lastly, attention must be called to the notable development of irregular wall-thickenings in the cells of the central tissue. Although similar thickenings occur in other regions of the meristem, they are more frequent and impressive in this zone. This appears particularly true of the apex of adventitious buds (pl. I, fig. 6). As seen in section view, the thickened areas are most prominent at the corners of a cell from which points they taper gradually or abruptly toward the lateral walls (fig. 2–12). When a cell of this type divides, the new walls are thin and delicate and hence easily distinguishable from the thicker lateral walls of the original "mother cell." This contrast in wall thickness obviously



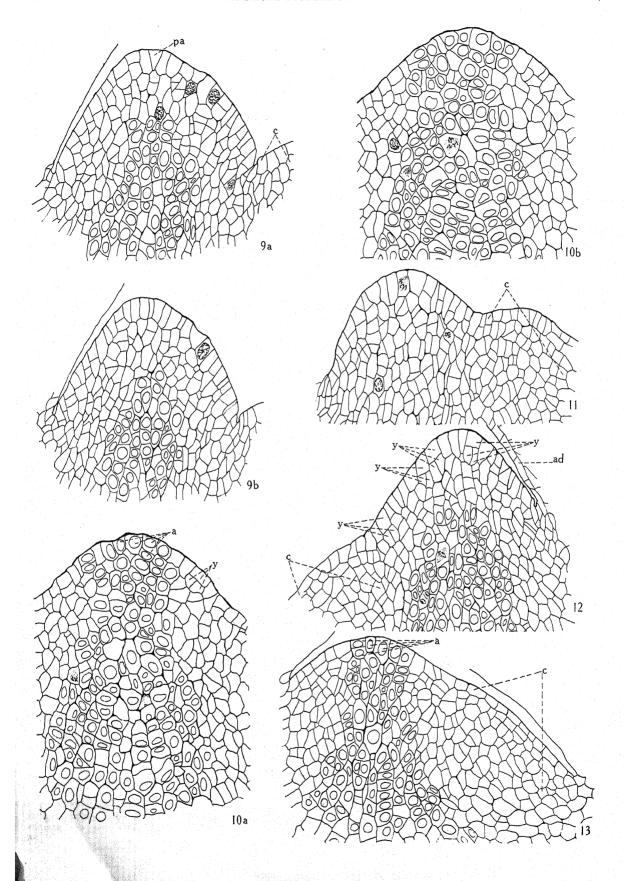
facilitates the determination of cell lineages within the central tissue. When observed in surface view, the lateral wall of a central cell typically appears as an irregular reticulum (fig. 6c-d; 7c, 10a). The unthickened areas in this meshwork presumably represent "primary pit-fields" from which the abundant simple pits, typical of the walls of the pith cells, take their origin. With the generous aid of Professor T. E. Rawlins of the Department of Plant Pathology, University of California, a brief study has been made to determine the structure and chemical nature of the thickened areas of the walls of cells in the central tissue. Our observations were made upon unstained longisections of the shoot apex of an adventitious bud of C. revoluta. When observed between crossed nicols, the outer layer of the lateral and corner thickenings was strongly birefringent or anisotropic, while the inner portion of the wall appeared isotropic or dark. After treating the sections with zinc-chlor-iodide followed by 65 per cent H<sub>2</sub>SO<sub>4</sub>, the lateral walls and corner thickenings in the meristem proper swelled and showed the blue color typical of cellulose. Using this technique, the primary pit-fields appeared as small light areas in the midst of a thicker blue reticulum. In the young pith cells, derived from the central tissue, the blue color was confined to the outer layer of the wall, the inner portion remaining colorless. Presumably then, the cell wall consists of an outer region composed of cellulose, while the inner portion may be pectic material or a combination of pectic material and non-pectic hemicellulose. These studies are to be regarded as exploratory in character, however, and no attempt can be made at this point to determine the limits between "primary wall" and "intercellular substance." A more detailed investigation of this problem is contemplated for the future.

THE PERIPHERAL TISSUE (zone III, fig. 1).—As seen in longitudinal section, this external tissuezone diverges from the apical initial group as a mantle of deeply-stained cells entirely surrounding the core of central tissue (pl. I, fig. 2, 4, 5; fig. 4c-g). Reference to figures 2-12 will demonstrate that the cells of the peripheral tissue show no evidence of a definite stratification at any point in the seedling apex. This is true even of the superficial cells which collectively bear no resemblance in their growth and planes of division to a "dermatogen" or 'tunica" layer. The present investigation therefore supports the earlier observations of Strasburger (1872), Warming (1877), and Bower (1884), all of whom emphasized that a typical "dermatogen" does not occur in the shoot tips of cycads. Under such circumstances, Chamberlain's (1935, p. 147, fig. 162) statement that in the shoot apex of the embryo of Dioon "periclines show that the dermatogen is not yet fully differentiated" (italics mine) seems vague and misleading. On the contrary, it seems extremely improbable to me that a dermatogen, in the sense originally defined by Hanstein (1868), is ever "differentiated" at any stage in the growth of the shoot apex in cycads (pl. I, fig. 6). Further comparative studies on other genera might provide exceptions to this conclusion. However, it seems significant that fundamental agreement with the situation in C. revoluta was noted in the apex of a vigorous expanding adventitious bud of C. circinalis L. as well as in the embryo, seedling, and adult plant of Zamia floridana (cf. fig. 13).

Aside from the irregular contributions made from the marginal cells of the central tissue-zone (fig. 6d, 7e, x), the bulk of the peripheral tissue clearly originates from the anticlinal derivatives of the apical initial group. These derivatives often attain a length of 25-30 microns and in their marked radial depth and lightly-stained appearance resemble "segments" of a prismatic apical cell (pl. I, fig. 2, 5; fig. 2a-b, 5b, 6c-d). As might be expected from their shape, these cells eventually divide periclinally, but the succeeding planes of division in the daughter cells are so rapid and variable that it becomes difficult to determine cell lineages with any confidence. It is evident, however, that the outer daughter cells produced by these earliest divisions retain a remarkable capacity for growth and cell formation throughout the further development of the peripheral tissue. The sequence of anticlinal, periclinal, and oblique divisions in these surface cells follows no fixed plan, and the radially-aligned cell groups are correspondingly variable in size and shape. Commonly, the periclinal division of a surface cell is followed by the anticlinal division of one or both of the daughter cells (fig. 5a, 6a, 7g). If the outer daughter cell divides first, a cell trio results in which the new intersecting walls have the form of a T. More frequently, however, the inner daughter cell is the first to divide. Such T-divisions are apparently very characteristic of C. revoluta and may occur at any point in the surface of the peripheral tissue (fig. 2a, 7f-g, 10a at y, 12 at y). In other instances, the original periclinal division of the surface cell may be followed by one or more divisions in the same plane in either or both of the daughter cells. This type of activity is sooner or later accompanied by anticlinal or oblique divisions in various cells of the group and results in a very complex cell net. Various stages in the process may be followed by comparing figures 3d and 8 with figures 9a-b.

It should be apparent from this description of cell formation that the peripheral tissue constitutes

Fig. 7-8. Longisections through shoot apices of seedlings of C. revoluta. Fig. 7a-g, series of sections illustrating the structure growth and extent of central and peripheral tissue at various levels in an apex. Fig. 7e-f, adjacent median sections showing irregular apical initial group at a; fig. 7a-d, respectively 60, 36, 24, and 12 microns at one side of fig. 7e. Fig. 7g, 18 microns at other side of fig. 7e. Note particularly the active propagation of peripheral tissue from the edge of the central tissue at a in fig. 7e-f and the primary pit-fields (p) in fig. 7c. Fig. 8, approximately median section of another apex illustrating a pseudoapical cell (pa) and a clear demarcation between central and peripheral tissue zones. All figures  $\times 217.5$ 



a zone typified by active centrifugal growth in thickness (fig. 1). This process reaches a maximum at the base of the shoot apex and is one of the factors which appears to determine the emergence of localized "folds" or leaf primordia. Although no effort has been made in this study to follow in detail the early development of either the foliage leaf or cataphyll, certain aspects of this problem deserve brief mention at this point. Firstly, the initiation, at least of the cataphyll, is evidenced by an acceleration of growth and division in the inner portion of the peripheral tissue accompanied by numerous periclines in the surface cells (fig. 11, 13). In this respect my observations are in general agreement with the statements of Strasburger (1872) and Warming (1877). But the emphasis of these investigators and of Bower (1884) on the early demarcation of a "dermatogen" layer in the young leaf is not supported by my study. An examination of radial longisections through a number of cataphyll primordia clearly showed that periclinal divisions occur abundantly in the surface cells, particularly near the adaxial basal region (fig. 2a-b). A thorough study of the significance of periclinal divisions, with particular reference to the processes of apical and marginal growth, is planned for the future. In addition, light may be thrown upon the vexed question (Thiessen, 1908) of the origin of the procambium and the development of the complex girdling leaftraces typical of cycads (cf. pl. I, fig. 1, 3).

Discussion.—The idea is widely held today that the cycads are an isolated and extremely primitive group of plants which show no obvious connection with either the ferns, other gymnosperms, or the angiosperms. Schuster (1932, p. 47) for example holds the opinion that the cycads are "the most primitive group in the Siphonogams without phyletic relationship to a definite group of higher plants." A somewhat similar viewpoint is maintained by Chamberlain (1935, p. 161), who states that the living cycads "are the last of their race, restricted in geographical distribution, restricted in number, and struggling for their very existence." If these views are correct, and there is considerable morphological evidence in their favor, no direct phylogenetic comparison can be made between the structure of the shoot apex in cycads and that of other living vascular plants. Furthermore, any effort at comparison must be restricted largely to the situation in Cycas revoluta, since previous studies on Zamia (Hofmeister, 1857), Ceratozamia (Dingler, 1882; Warming, 1877), and other species of Cycas (Warming, 1877; Bower, 1884) are both vague and contradictory. Under these circumstances, the following anatomical comparison is admittedly provisional in character. Its chief values may consist (1) in directing attention to the value of meristem structure in problems of comparative morphology and (2) in indicating the need for extensive studies on the shoot apex of all the major groups of the Tracheophyta.

A comparison between the structure of the shoot apex in cycads and Ginkgo should be of particular interest in view of certain resemblances in reproduction and embryogeny between these primitive gymnosperms (Chamberlain, 1920, p. 149). Although Ginkgo agrees with C. revoluta in the fact that all tissues of the apex originate from a single apical initial group, the zonal structure of the terminal meristem in the two genera is quite dissimilar. This is shown in the first place by the contrast in structure and growth of the median axial region of the shoot apex. In Ginkgo, as I have recently described in detail (Foster, 1938), the upper median region of the apex consists of a clearly-defined cupshaped zone of enlarged, slowly-dividing "central mother cells." The formation of the rib-meristem from the base of this cup involves a rapid and abrupt transition to a zone of much smaller, more deeplystained and more actively-dividing cells. Depending upon whether a "spur" or "long" shoot is examined, there is a short or prolonged period of growth in the rib meristem which ultimately differentiates into the pith of the shoot axis (cf. Foster, 1938, fig. 1-2 and pl. 25, 26, 27). In marked contrast, there is no comparable demarcation of successive vertical zones in the shoot apex of C. revoluta. Here, a prominent but continuous core of central tissue arises from a few subterminal cells and produces basally the broad pith of the shoot axis (pl. I, fig. 1). In other words, there is no sharp boundary in Cycas between an upper "mother cell region" and a lower zone of rib meristem. As I have already shown, more or less prominent vertical series of cells, resembling rib meristem, may form at any level in the central tissue, although they are most obvious in the basal portion (pl. I, fig. 2, 5, 6; fig. 2, 3, 5-10, 12). A second interesting contrast between Cycas and Ginkgo

Fig. 9-13.—Fig. 9-12. Longisections through shoot apices of seedlings of C. revoluta. Fig. 9a-b, adjacent sections slightly distal to median plane of apex, illustrating centrifugal growth in thickness typical of peripheral tissue. Note prominent pseudoapical cell (pa) and the irregular sequence of periclines and anticlines in its apparent surface derivatives in fig. 9a.—Fig. 10a-b, adjacent median sections of an unusually large apex, illustrating the origin and active growth of the massive central tissue. Note particularly the irregular files of rib-meristem in the lower portion of these sections. A typical T-division in a surface cell is shown at y in fig. 10a.—Fig. 11, non-median section of another apex illustrating the active periclinal division of surface cells during cataphyll initiation. Note the obliquely oriented metaphase in a "terminal" surface cell.—Fig. 12, non-median section of another apex illustrating typical structure and growth of the peripheral tissue. The cell-groups labelled y have arisen by T-divisions. Note smaller cell size in central tissue as compared with fig. 10a-b.—Fig. 13. Approximately median section through the shoot apex of a seedling of Zamia floridana. Note the large size of several of the cells in the central tissue, the active periclinal division of surface cells and the early phases of cataphyll initiation on the right flank of the apex. Legend: a, cells of apical initial group; ad, adaxial surface cells of cataphyll primordium; c, cataphyll initial; pa, pseudoapical cell; y, cell groups originating by T-divisions. All figures  $\times 217.5$ .

is provided by the structure and mode of growth of the peripheral tissue of the apex. In both genera, this zone exhibits marked "embryonic" characters as shown by the numerous mitoses and the deeplystained appearance of the cells. Aside from this resemblance, cell growth and cell arrangement are noticeably different, particularly in respect to the behavior of the superficial cells. In Ginkgo, these cells tend to divide frequently in an anticlinal plane and hence appear collectively as a more or less discrete "surface layer" (Foster, 1938, fig. 5-12). On the other hand, the surface cells of the apex of C. revoluta vary so greatly in their size, shape, and successive planes of division that it is impossible to recognize any regularity of arrangement. This contrast appears to reflect a basic difference in the general distribution of growth throughout the peripheral tissue of Cycas and Ginkgo. In the latter, surface growth accompanied by a tendency toward a stratification of cells is characteristic (Foster, 1938, fig. 3), whereas in Cycas, centrifugal growth in thickness results in a highly variable pattern of cells (fig. 1).

From a comparative standpoint, the terminal meristems of Ginkgo and Cycas revoluta represent two well-defined "types" which permit of no close comparison with the shoot apices of other living vascular plants. Probably the closest resemblance to Cycas is shown by certain of the Abietineae. A median longisection through the apical cone of a vigorous expanding shoot of Abies venusta (Dougl.) K. Koch. will serve as an example (pl. I, fig. 7). It is evident from this photomicrograph that a central core of vacuolating, tannin-filled cells is surrounded by a peripheral zone composed of three or four "layers" of small densely-cytoplasmic cells. These two histological regions obviously show a noticeable similarity respectively to the peripheral and central tissue-zones in the shoot tip of C. revoluta (pl. I, fig. 2, 5, 6). Differences nevertheless will soon be apparent if the size and particularly the arrangement of the cells in the two zones of Cycas and Abies are compared. Clearly, the cells of the peripheral tissue in Abies are more or less definitely stratified as contrasted with the irregular cell pattern in the presumably equivalent zone in Cycas. Furthermore, the boundary between the central and peripheral tissue zones is "sharp" in Abies, while, as I have previously emphasized, only approximate limits can be set between these zones in C. revoluta. This latter difference appears to be correlated with a greater regularity in the direction of growth and plane of division of the central tissue in Abies which exhibits more uniformly the general features typical of rib meristem. If, as I have previously suggested (Foster, 1938, p. 553), the two major tissue regions in the shoot apex of Abies and other conifers (cf. Koch, 1891; Korody, 1937) represent a "transitional stage" in the evolution of the zonation characteristic of the angiosperms, the situation in Cycas acquires considerable interest. That the peripheral tissue in this species should be regarded as typify-

ing an extremely "primitive stage" in the evolution of the tunica zone (Schmidt, 1924) in angiosperms might readily be questioned from the standpoint of current phylogenetic theory. The morphological interest of the present study on Cycas is that it gives us, to some degree at least, an idea of the nature of terminal growth in one of the most ancient types of existing seed plants. Whether the growth and associated cellular organization of this shoot apex will eventually aid in the interpretation of the apical meristems of more advanced seed plants or whether these features are unique and peculiar to cycads are problems which must await further comparative study for their solution.

#### SUMMARY

A detailed study has been made of the structure and growth of the shoot apex of seedlings and adventitious buds of *Cycas revoluta* Thunb. The anatomy of the terminal meristem of the seedling of *Zamia floridana* A.DC. is also briefly described.

No evidence has been found of the existence of a "permanent" apical cell. On the contrary, all the tissue of the shoot apex in both Cycas and Zamia can be traced in ultimate origin to the activity of a single group of apical initial cells which divide anticlinally, periclinally, and obliquely without regular sequence. Although a large prismatic cell may appear to dominate for a time in this initial group, its individuality is eventually submerged as a result of irregular and variable planes of segmentation.

The tissue derived from the apical initial group in Cycas is not uniform in structure but is segregated into two more or less clearly demarcated zones: (1) a prominent conical zone of central tissue which is completely surrounded by a mantle of deeply-stained peripheral tissue. These zones are characterized by significant differences in the growth, planes of division, arrangement, and relative size of the component cells.

Pronounced cell-enlargement, followed by the formation of well-defined cell groups, are the distinctive histogenetic features of the central tissue in Cycas. When the direction of growth and enlargement of a given "mother cell" is approximately vertical, a linear series of daughter cells is produced. This type of cell enlargement and cell formation is particularly evident in the lower region of the central tissue, where the irregular filamentous cell groups collectively resemble a rib meristem. At any position in the central tissue, however, cells may enlarge more or less uniformly in all directions, and the resulting cell lineages are correspondingly difficult to interpret. The walls of the central cells, especially at the corners, are often prominently thickened. A preliminary study of these thickened areas in an adventitious bud of C. revoluta between crossed nicols showed that the outer lamellae are strongly birefringent (i.e., anisotropic), while the inner region appears dark (i.e., isotropic). When treated with chlor-zinc-iodide followed by 65 per

cent H2SO4, the outer portion of the thickenings gave the blue color typical of cellulose.

The peripheral tissue of the apex in Cycas is composed of smaller cells characterized by their "embryonic" appearance (i.e., absence of impressive enlargement) and frequent divisions. Judging from the cell net and the orientation of mitotic figures, the prevailing direction of growth in this tissue-zone is centrifugal. The surface cells retain a marked capacity for continued growth and division; collectively, they do not resemble in arrangement or plane of division a "dermatogen." Periclinal divisions in these surface cells are followed usually by the anticlinal division of one or both of the daughter cells. Often several successive periclinal divisions, followed by anticlines or obliquities in the derivatives, may occur. The radially-aligned cell groups thus formed are complex in structure and variable in size. Foliar structures are initiated at the base of the shoot apex by a localized acceleration of growth and cell division in the peripheral tissue, accompanied by frequent periclines in the surface cells. Periclines are also numerous in the adaxial surface cells of the cataphyll primordia, which, at least during early ontogeny, do not possess a typical dermatogen.

An anatomical comparison of the shoot apices of Cycas revoluta and Ginkgo biloba reveals few points of resemblance, and the terminal meristems of these forms are regarded as distinct "types" among living vascular plants. The closest structural resemblance with Cycas is exhibited by certain conifers such as Abies venusta.

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# THE COLOR OF MYXOMYCETE PLASMODIA 1

Paul E. Kambly

ALTHOUGH THERE is considerable evidence in the literature on myxomycetes that plasmodia of some species exhibit consistency of color on different substrata, there are also many conflicting reports concerning the plasmodial color of other forms. The Lister monograph (1925) and, to a much less extent, Macbride and Martin (1934) use plasmodial color as a taxonomic character. Pinov (1908) postulated sexual dimorphism in Didymium nigripes on the basis of the occurrence of differently colored plasmodia. Skupienski (1934) reported a thermophilic race of Didymium xanthopus which is distinguishable, in part, by a white plasmodium. A. Lister (1888) was one of the first to describe a color change due to the ingestion of colored particles by a plasmodium. Cadman (1931) and Gray (1938) mention the same phenomenon. Color changes due to bacterial associates have been described by Pinov (1903) (1915), Skupienski (1920), and Raper (1937). If plasmodial color is to hold any place as a taxonomic character or as an aid in distinguishing strains and races, information concerning the factors which influence color is essential. This paper reports observations on plasmodia grown in the laboratory, with particular reference to color variations caused by certain bacterial associates.

MATERIALS AND METHODS.—The plasmodia used in the experiments described were collected in the field or obtained from spore sowings. Both sclerotia and active plasmodia were collected and placed in moist chambers in the laboratory. Cultures obtained from spores were started by sowing spores in distilled water in Syracuse watch glasses. The watch glasses were left on the laboratory table and no precautions taken to protect the swarm-cells from light. They were kept covered by another watch glass to reduce the risk of contamination by another myxomycete. The spores were observed from time to time with either a low power objective or a water immersion lens and after germination were transferred to nutrient agar with a clean pipette. The chief advantage of sowing spores in water rather than directly on the nutrient agar is that viability of the spores can be determined in water and that failure to obtain plasmodia because of non-viable spores may be ascertained. A further advantage is that growth time on agar is shortened, and thus the amount of contamination is reduced. A 2.5 per cent carrot decoction agar proved to be a very satisfactory nutrient medium for obtaining plasmodia from spores. The amount of available nutrient material in this medium seemed to be small enough to discourage excessive mold growth and yet great enough to support the growth of a bacterial film over the surface of the agar. This nutrient agar was pre-

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pared by boiling the carrots for one and a half to two hours. Then agar was added, the carrot particles were removed by filtration, the pH adjusted to approximately 7, and the medium was tubed and autoclayed.

Serratia marcescens, Chromobacterium violaceum, and Sarcina lutea, grown on beef-peptone agar, and Phytomonas fascians<sup>2</sup> grown on potato-dextrose agar were used as plasmodial associates. These organisms produced red, violet, yellow, and cadmium yellow pigments, respectively. In experiments performed with plasmodia collected in the field the plasmodia were first obtained in active condition upon plain agar plates and the bacteria added as a suspension in distilled water. Plasmodia obtained from spore sowings were kept on nutrient agar, and the bacterial suspensions were added. All cultures were maintained in the diffuse light of the laboratory at room temperatures which varied from 20°C.

The arrangement and nomenclature which follows is that of Machride and Martin.

DETAILED OBSERVATIONS.—Records of several Fuligo septica plasmodia collected in the field, indicate a variability in plasmodial color in this species. These plasmodia fruited and were positively identified. One specimen growing on apple bark had an ocher-gray plasmodium when collected, but transplants to plain agar plates yielded both white and yellow plasmodia. The yellow forms tended to become white in the laboratory. A different specimen produced only white transplants. Another plasmodium growing on very rotten wood was bright yellow when collected. Three days later part of this collection on plain agar was snow white. On the same day one culture plate containing wood on plain agar showed a greenish yellow plasmodium, and part of the same plasmodium in another Petri dish on plain agar was light yellow and "frothy." Six days later this light yellow plasmodium had fruited. Transplants of all three collections were subjected to suspensions of Serratia marcescens and always became pink within a few hours. None of these completed its life cycle, and sclerotia were formed in every case. Suspensions of Sarcina lutea produced no noticeable color change but the plasmodia remained active slightly longer than with Serratia. White plasmodia of Fuligo septica have been obtained from spore sowings on 2.5 per cent corn agar, but none grew to large size nor completed its life cycle in culture.

Physarum polycephalum.—Since it was possible to collect the plasmodia of this species in the field, no attempts were made to obtain them from spore sowings. Several plasmodia found growing on old

<sup>2</sup> Tilford, P. E. 1936. Fasciation of sweet peas caused by *Phytomonas fascians* n. sp. Jour. Agric. Res. 53: 383-394. Culture obtained from Dr. E. M. Hildebrand of Cornell University. hymenophores of *Pleurotus* were all deep yellow. Three different plasmodia collected from rotten logs were definitely greenish yellow. Transplants of these plasmodia to plain agar became orange to redorange within a few hours after a suspension of *Serratia marcescens* was added. In one case a transplant from a yellow plasmodium formed sporangia while associated with the red bacteria. The hypothallus and stalks of these fructifications were red, in contrast with the typical pale yellow of the corresponding parts of sporangia formed on plain agar without bacteria. Suspensions of *Sarcina lutea*, *Chromobacterium violaceum*, and *Phytomonas fascians* neither stimulated growth nor caused changes in color.

Physarum compressum.—Grav reported the cultivation of this species on corn decoction agar, but he experienced difficulty in maintaining transplants. A 2.5 per cent carrot agar proved to be an excellent nutrient medium for the plasmodia obtained from spore sowings. Transplants on this medium enlarged rapidly, produced characteristic sporangia, were consistently pearly white, and did not change color before the formation of sporangia. When Serratia marcescens was added to cultures, the plasmodia became pink and continued their growth. Plasmodia associated with Chromobacterium violaceum, Sarcina lutea, and Phytomonas fascians continued growing but did not change in color. Characteristic sporangia were formed with all four species of bacteria. The stipes were ashen in color in all cultures except those which contained Serratia. These were first red and then brown. Both of these colors occur in natural developments of the species.

Physarum bivalve.—The white plasmodia of this species were obtained easily on carrot decoction agar, and typical bilabiate fructifications were formed by most of them. The typical white plasmodia showed a greater tendency to change color than those of most of the species studied. Alternaria contaminants present in some plates caused the plasmodia to become darker in color because of the ingestion of spores. These large muriform spores could be seen under the binocular as they were carried by the streaming protoplasm. This is in contrast with Howard's (1931) observations of Physarum polycephalum which he found did not ingest Alternaria spores. However, since the plasmodial trail was strewn with the spores it is doubtful whether they were a source of food. The addition of a suspension of Serratia marcescens caused the plasmodia to become pink. In a few cases plasmodia associated with Chromobacterium violaceum exhibited a faint violet tinge. Sarcina lutea caused the plasmodia to develop a faint yellow tint. Plasmodia remained white when grown in the presence of Phytomonas fascians.

Physarum didermoides.—Carrot decoction agar promoted some growth of this species, but none of the plasmodia flourished or completed its life history. The plasmodia were white when small but showed a tendency to develop a yellow tint as they

enlarged. Serratia marcescens was the only bacterial associate which caused a change in plasmodial color, and with this species the plasmodia were pink. Chromobacterium violaceum, Sarcina lutea, and Phytomonas fascians did not encourage growth nor affect the color of the plasmodia.

Physarum leucophaeum.—Only one collection of the plasmodium of this species was obtained. It was found growing on the inside of an old hollowed turnip, and the only fructifications obtained were formed on the original turnip substrate. The plasmodium was yellow on the turnip and remained yellow on plain agar. Carrot agar was not a suitable culture medium, causing a rapid bunching of the plasmodia followed by disintegration. Since portions of the original plasmodium did spread out into typical plasmodia on plain agar plates, it was possible to study the effect of the bacterial associates. Serratia marcescens caused the plasmodium to become slightly orange but also resulted in a rapid withdrawal of the protoplasm into the larger veins. After withdrawal the protoplasm did not resume activity and soon deteriorated. Chromobacterium violaceum, Sarcina lutea, and Phytomonas fascians caused no color changes but did affect the plasmodia in the same way as Serratia marcescens.

Physarum sp.—The plasmodia of this form appeared as contaminants on carrot decoction agar on which spores of Enerthenema papillatum had been sown. They were colorless and watery in appearance, and the fructifications formed were anomalous plasmodiocarps. Serratia marcescens caused the plasmodia to become pinkish red. No color changes were evident when plasmodia were associated with Chromobacterium violaceum. Both Sarcina lutea and Phytomonas fascians caused the plasmodia to develop a yellow tint which became more intense as the plasmodia bunched to form fructifications.

Physarella oblonga.—One collection of the plasmodium of this species was made from a rotten log. It was greenish yellow and maintained this color on carrot decoction agar as well as 2.5 per cent corn decoction and 5 per cent hay extract agar. The only sporangia produced in culture formed on plain agar to which small portions of the original wood, bearing part of the plasmodium, had been added. Serratia marcescens added to a plasmodium on plain agar caused it to become reddish orange. Chromobacterium violaceum, Sarcina lutea, and Phytomonas fascians caused the plasmodial portions to bunch quite rapidly. This bunching resulted in an increase of the intensity of the yellow color.

Didymium squamulosum.—The plasmodia of this species were obtained on 5 per cent carrot, 2.5 per cent carrot, 3 per cent hay, and 2.5 per cent corn decoction agars. Young plasmodia were white, but older plasmodia varied. Large plasmodia on carrot decoction agar were reddish brown. Plasmodia creeping over molds were frequently violet-brown to reddish brown because of the ingestion of spores. Characteristic fructifications were formed on carrot agar. Serratia marcescens caused the plasmodia

to become pink. Chromobacterium violaceum, Sarcina lutea, and Phytomonas fascians did not cause any evident color change. None of these plasmodia fruited while associated with the colored bacteria.

Didymium nigripes.—Plasmodia formed on carrot decoction agar were always white when young. Older plasmodia growing on carrot agar often appeared light yellow to carrot colored. This observation agrees with that of Cadman on the same species. Plasmodia associated with Serratia marcescens became pink but did not complete their life histories. Chromobacterium violaceum, Sarcina lutea, and Phytomonas fascians caused no color changes. Plasmodia associated with these species exhibited the same color variations as those grown on carrot agar to which the pigmented bacteria had not been added.

Didymium xanthopus.—Plasmodia of this species were obtained easily on 2.5 per cent carrot agar. On several occasions they appeared as contaminants in cultures of other plasmodia. Young plasmodia were gravish white but soon changed to a carrot color which they maintained until fruiting. Occasionally the color changed to a pinkish fawn just before sporangia began to form. Transplants of plasmodia to fresh carrot decoction plates thrived and formed numerous characteristic sporangia. The stipes of freshly formed sporangia were much lighter in color than the stipes of the collection from which the spores were sown. Several different plasmodia were grown on carrot agar with Serratia marcescens. These became pink to red within a few hours. Sporangia formed by these plasmodia had pink stipes and heads when young. The older heads appeared characteristic for the species, and the stipes became a dark reddish brown in contrast with the lighter color of stipes formed on carrot agar to which no bacteria were added. Plasmodia on carrot decoction agar to which a suspension of Chromobacterium violaceum was added remained carrot colored until fruiting. Sarcina lutea and Phytomonas fascians suspensions caused no color change, but the plasmodia grew extremely well in association with these bacteria. Stipes of the fruiting structures formed in association with Sarcina and Phytomonas were much lighter in color than those formed in association with Serratia and Chromobacterium.

Clastoderma Debaryanum.—This species was collected in the sclerotium stage. Active plasmodia appeared about six weeks after the sclerotia were placed in a moist chamber. These plasmodia were greenish yellow when crawling over plain agar but were deeper yellow to light brown on wood. No fructifications were formed from transplants to nutrient agar unless a piece of the original pine wood was included with the transplant. Most of the scattered sporangia appeared on the wood, but several were formed on the nearby agar. All attempts to obtain plasmodia from spore sowings were unsuccessful. The only reference to the color of the plasmodium of this species other than in Lister's monograph is one by Beardslee (1908). Lister gives the

color as watery white. Beardslee states, "a log was found and kept under observation which seemed to be completely filled with its plasmodium. This appeared at frequent intervals, covering the log with a pale yellow network of plasmodial threads, changing over night to a thick growth of the delicate sporangia." Adding a suspension of Serratia marcescens to the greenish yellow plasmodia on plain agar resulted in orange-colored plasmodia. Chromobacterium violaceum, Sarcina lutea, and Phytomonas fascians caused no noticeable change in color. As was previously stated, these transplants did not finish their life history. In most cases the plasmodia bunched up and disintegrated, but they occasionally formed sclerotia.

Ophiotheca Wrightii.—Plasmodia appeared on leaves in a moist chamber. They varied in color through white, gray, and light brown. The darker colored plasmodia were those growing on the decaying leaves. On plain agar the plasmodia were white. Serratia marcescens caused the white plasmodia on plain agar to become pink and then to clump into a reddish knob. Chromobacterium violaceum, Sarcina lutea, and Phytomonas fascians did not encourage plasmodial growth nor cause changes in color. In each case the plasmodium bunched up into a small mass. The fructifications obtained were all formed on the natural substrate of dead leaves.

Hemitrichia stipitata.—The plasmodium of this species was yellow when collected but varied from light yellow to a marked greenish yellow in the moist chamber. It was active for three months before any sporangia were formed. Transplants to 5 per cent corn decoction agar varied from white to deep yellow. Several plasmodia on 2.5 per cent carrot decoction agar were cream colored. Plasmodia associated with Serratia marcescens became deep orange colored but withdrew into the coarse veins very rapidly and did not resume activity. The same type of bunching was evident after the addition of Chromobacterium violaceum, Sarcina lutea, or Phytomonas fascians.

Unidentified plasmodia.—In addition to the plasmodia of the species listed above, twenty-five other plasmodia collected in the field, but which failed to form fructifications, were used in this study. Two of these were white when collected, eight were yellow, and fifteen were greenish yellow. The greatest variation in color was shown by one of the two white collections. This produced white, yellow, and greenish yellow transplants. The stock plasmodium growing on wood in a moist chamber became greenish vellow and maintained this color for the four months it was actively growing. There was some fluctuation between yellow and greenish yellow in other plasmodia, but in most cases this variation was associated with, and perhaps a result of, bunching or spreading out of the plasmodium. All twenty-five of these plasmodia changed in color on plain agar plates to which suspensions of Serratia marcescens were added. The color varied from pink through orange to red. No color changes were evident in any

case as a result of adding suspensions of Chromo-bacterium violaceum, Sarcina lutea, or Phytomonas

Discussion.—From the above observations, it is evident that the plasmodial color of some species of myxomycetes is extremely variable. The chemical nature of the pigment has not been determined. Zopf (1889) believed that the yellow pigments are identical with the lipochromes of animals. In addition to the lipochromes, each species he studied possessed at least one other pigment which was soluble in water. Solacolu (1932) concluded that pigments extracted from the sporangia of twenty-six species were anthracene derivatives. Seifriz and Zetzman (1935) concluded: "1. The yellow pigment of the slime-mould Physarum polycephalum is an acidalkaline indicator with a color range from deep red orange to bright yellow-green, and a corresponding pH range from 1 to above 8. 2. Within the slimemould the pigment serves as an acid-alkaline indicator and shows the plasmodium to undergo changes ranging from pH 8 when fruiting to pH 1.6 (possibly 1.2) when a sclerotium is formed. 3. The suggestion is made that the yellow pigment of slimemoulds belongs to the group of respiratory ferments known as flavones, lyochromes, or flavins.' On the assumption that pigments are pH indicators, one can readily account for the gradation between yellow and green which many of the plasmodia used in this study exhibited. The suggestion that the vellow pigment is a respiratory ferment offers a further explanation for variations in color from yellow to white. However, there is little experimental evidence to support this latter view.

The color change produced in every case when plasmodia were associated with Serratia marcescens was similar to that described by Raper (1937) for the pseudoplasmodia of Dictyostelium discoideum. The red bacterial pigment is contained within the cells and is not water soluble. The plasmodia used in this study seemed to ingest and digest part of the bacterial cells but evidently could not digest the pigment, which seemed to collect in scattered vacuoles in the plasmodia and was not excreted when the plasmodia fruited. The failure, with two

exceptions, to obtain corresponding effects with Chromobacterium violaceum, Sarcina lutea, and Phytomonas fascians cannot be satisfactorily explained at the present time. Watanabe (1932) found that Sarcina lutea and Chromobacterium violaceum (B. violaceus) were generally a more adequate source of food for plasmodia than Serratia marcescens (B. prodigiosus). His work shows that the bacteria are ingested. The most plausible explanation for lack of coloration seems to be that the bacterial pigments are broken down by the digestive processes in the plasmodia.

#### SUMMARY

Myxomycete plasmodia collected in the field exhibited considerable variation in color when maintained in moist chamber cultures in the laboratory.

Thirty-nine different plasmodia, including those of Fuligo septica, Physarum polycephalum, Physarum compressum, Physarum bivalve, Physarum didermoides, Physarum leucophaeum, Physarella oblonga, Didymium squamulosum, Didymium nigripes, Didymium xanthopus, Clastoderma Debaryanum, Ophiotheca Wrightii, and Hemitrichia stipitata, changed color when associated with Serratia marcescens. With the exception of Physarum bivalve and Physarum sp., none of these plasmodia was visibly affected by either Chromobacterium violaceum or Sarcina lutea. Only the colorless watery plasmodium of Physarum sp. changed color because of association with Phytomonas fascians.

Young sporangia of Physarum polycephalum, Physarum compressum, and Didymium xanthopus formed in association with Serratia marcescens were pink to red.

The laboratory cultivation of Physarum bivalve

is reported for the first time.

The wide variation in color under natural and experimental conditions suggests that the color of a plasmodium is not a good taxonomic character nor a dependable factor in the delimitation of strains or races.

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## THE RELATION OF SPECIFICITY OF ORCHID MYCORRHIZAL FUNGITO THE PROBLEM OF SYMBIOSIS $^{\scriptscriptstyle 1}$

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THE EXISTENCE of a mutually beneficial symbbiosis between orchids and certain filamentous fungi has been inferred for a number of years. Two principal arguments have been offered in support of this symbiotic view, both of them dealing with the relation of the fungi to the germination of the orchid seeds. The main thesis was advanced by Bernard in 1904 and subsequently supported by Burgeff (1909, 1911, 1936), Costantin (1917), and Ramsbottom (1922, 1927). They maintained that orchid seeds would not germinate without the aid of endophytic fungi found in the roots of the orchids. The second argument dealt with an alleged specific relationship between the orchid and the fungus species and was also advanced by Bernard (1909), who claimed that different orchid plants of the same species or the same genus would always harbor the same species of fungus. This view was held as late as 1927, when Ramsbottom stated that "The same fungus is always present in Cattleya roots and is distinguishable in culture, even with the naked eye, from a culture of the fungus from Odontoglossum."

Certain of Bernard's results led him to apply this idea of specificity to the phenomenon of symbiotic germination. He stated that the seeds of one species or genus of orchids would germinate only in the presence of the fungus from that orchid or others of closely related genera.

Other arguments favoring the existence of a mutually beneficial relationship between orchid and fungus are concerned with the effect of the fungus on the developing seedling or the mature plant. Bernard thought that infection of orchid seedlings led to a tuberization or secondary thickening of stems and rhizomes. Other possible benefits that the orchids derive from their fungal symbionts have been advanced by many workers. Most commonly held is the belief that the fungus serves as a means of absorption and conduction of water and nutrients from the substrate to the roots. This is probably a carry-over from early theories as to the function of ectotrophic fungi on the roots of woody plants. Actually the connecting hyphae between root and soil are very rare in the endophytic forms and prob-

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ably serve only as agents of infection. Early workers thought that the lack of root hairs in many orchids necessitated the postulation of some other absorbing surface. As a matter of fact, all terrestrial orchids are supplied with root hairs and all epiphytic orchids produce them under conditions of sufficient moisture. The lack of root hairs on some roots of epiphytes is a condition peculiar to the velamen-covered aerial roots, and these aerial roots are almost constantly devoid of mycorrhizal fungi.

Other investigators have thought that plants produced asymbiotically were "abnormal" and would not flower in the absence of fungi in their roots. All these theories concerning the obligate nature of symbiosis, except the one concerning specificity, were disproved by Knudson (1930) when he recorded the production of a normal flower on a Laeliocattleya hybrid grown asymbiotically on a sterile mineral nutrient solution lacking carbohydrates.

SEED GERMINATION.—Early practical orchid growers obtained germination of orchid seeds only by sowing them on a compost containing the living parent plant. Link (1840) was the first to demonstrate that orchid roots may contain an endophytic fungus. This root infection was shown to be fairly widespread by Wahrlich (1886), who found fungi in the roots of more than 500 species of orchids. Bernard (1899) studied very young seedlings (protocorms) of Neottia nidus-avis in nature and found them to harbor a fungus also. From these facts he formulated the hypothesis that the endophytic fungi in the roots could cause germination of the seeds. He proceeded to test this by isolating fungi from orchid roots, growing them in pure culture, and sowing seeds on the pure cultures. He isolated a large number of filamentous fungi from the roots of a Laeliocattleya hybrid and planted seeds of the same hybrid on pure cultures of each of the fungi. With one fungus he obtained good germination. This was proof of his theory that fungi from orchid roots can cause germination of seed. However, Bernard also concluded that because one particular fungus induced germination, it was therefore necessarily the true symbiotic fungus for the orchid in question, and the others were merely contaminating organisms. This conclusion has served as the criterion of a symbiotic orchid fungus—i.e., one which occurs in the roots of an orchid and, in addition, is able to

induce germination of that orchid's seeds. Bernard (1909) and Burgeff (1909) elaborated the early results and concluded that germination can be brought about only by the aid of endophytic fungi belonging to the genus *Rhizoctonia*.

Knudson (1922) showed, in a series of well-executed experiments, that seeds of several representative orchid genera would germinate in the absence of any fungus, if they were provided with soluble sugars. He further showed that the presence of sugar was essential only in the primary stages of germination and that the plants were able to grow normally on a medium containing only the essential mineral elements as soon as the first leaves were formed. He held that the fungus acted through enzymatic action on the starch in the external environment and through an increase of the acidity of the medium

Additional proof that germination of orchid seeds is not dependent on the action of an endophytic fungus was offered in a series of observations on symbiotic germination by fungi other than orchid Rhizoctonias. Burgeff (1909) found that an undetermined brown saprophytic fungus (not a Rhizoctonia) occurring as a contaminant in one of his cultures of Laeliocattleya Acis produced marked stimulation of growth of the seedlings. A white bacterium occurring as a contaminant in other cultures also produced this effect. Knudson recorded the occurrence of germination in asymbiotic cultures contaminated with saprophytic fungi (Penicillium sp.), and most other workers in the field have noted instances where chance organisms have produced marked stimulation of growth. Knudson (1925) tested the extent of this phenomenon by planting Cattleya seeds with an orchid fungus (Rhizoctonia repens). He found that Phytophthora sp., Choanophora sp., and Cucurbitaria sp. all produced germination about as efficiently as did the orchid fungus.

Burgeff (1932) found species of Marasmius, Xerotus, Fomes, and other basidiomycetes to be effective in producing germination of seeds of several Malayan saprophytic orchids. Cappelletti (1935a) obtained good germination of a Cymbidium Cibele hybrid with the fungi Pleurotus, Hypholoma, and Corticium. Derx (1937) pointed out that all regularly accepted orchid endophytes are basidiomycetes. He tested the possibility that other basidiomycetes could cause germination by using Corticium octosporum isolated from garden soil. He found it to cause germination in Laeliocattleya and Dendrobium.

In order further to test the extent of these observations, a series of cultures was set up in the present investigation in which seeds of Cordula insigne were sown with various fungi. Medium Sb of Burgeff (1936) was used as a substrate. After five months the following results were obtained. Good germination occurred with: Ceratostomella sp., Pleurotus ostreatus, and Corticium Catonii; poor germination with Chaetomium bostrychodes and Rhizoctonia repens from Goodyera pubescens; and

no germination with Sporormia leporina, a Chaetomium from Cymbidium, and an unidentified mycorrhizal fungus from Epigaea repens (Phoma sp.?).

The induction of germination by such a wide range of organisms would indicate that there is nothing very exacting about the requirements of the orchids. Perhaps the most significant discovery concerning the physiological relations of fungi and orchids was the observation by Burgeff (1934) and Cappelletti (1935a) that the heat-killed mycelium of an appropriate fungus would cause as good germination as would the living mycelium. This discovery led to the view that the fungus might produce some chemical compound necessary for germination. Burgeff and Cappelletti independently proceeded to identify this growth-promoting substance, whatever it might be. Burgeff isolated from the killed mycelium a substance which he thought related to bios or vitamin B and which stimulated the germination of seeds of the Sarcanthinae in the absence of a living fungus. Cappelletti (1935b), by comparing the effects of the fungi with those of known compounds, concluded that vitamin C was necessary for germination of Cymbidium seeds. Both these workers used seeds which are in some cases difficult to germinate by the non-symbiotic method. Both Vanda and Phalaenopsis (Sarcanthinae) and Cymbidium (Cymbidiinae) will give excellent non-symbiotic germination if the seeds are fresh, but with old seeds the presence of a fungus is necessary for good germination. It is presumably only in these cases that the presence of some growthpromoting substance is necessary.

A summary of these results indicates that orchid seeds will germinate without the aid of any fungus or other micro-organism, provided they are supplied with an adequate mineral nutrient solution and a suitable sugar at the proper hydrogen-ion concentration. If they are planted on an unfavorable medium, such as one containing starch, they need the aid of some fungus or other agency which can change the starch to sugar and can maintain the proper pH. In case this agent is a living organism, it is also necessary that no toxic substance be produced or that the amount of growth of the organism be such as physically not to smother the seeds. This organism need have no relation to the mature orchid plant and is definitely not necessarily the fungus found in the roots of the mature plant. In most cases, fungi fulfilling these requirements are basidiomycetes, but, in several instances, members of the oomycetes and of the ascomycetes have proved capable of inducing germination, and it is probable that many more, especially ascomycetes, will be found which will cause germination.

In nature it is possible that soil fungi capable of changing insoluble complex carbohydrates to simple sugars play a large part in orchid seed germination by providing a suitable food supply in the soil. Burges (1936) has shown that an endophytic fungus isolated from *Orchis incarnata* was able to increase the free, water-soluble carbohydrate content

of previously sterilized fen soil, and a similar action is known to be brought about by such common soil saprophytes as the Mucors and the Penicillia. As Burges points out, "The soil organisms responsible for the decomposition (of insoluble carbohydrates) bear no necessary connection with mycorrhizal fungi, but it is recognized that a fungus may conceivably act in both ways." In any case, there has been no proof that orchid Rhizoctonias alone are responsible for germination in nature.

The above conclusions with reference to the necessity of fungi for germination are applicable only to green, autotrophic orchids. The purely saprophytic forms have been insufficiently studied as yet to offer any firm basis for conclusions.

In the present investigation an effort was made to isolate as many strains or species of mycorrhizal fungi, especially Rhizoctonias, as possible from orchid plants growing in different ecological habitats or different localities in Wisconsin. In addition, isolations were made from orchids secured from Costa Rica and Mexico, for purposes of comparison. The Mexican species were supplied through the kindness of Mr. Eric Ostlund of Cuernavaca, Mexico. Plants of Spiranthes cernua were obtained from Mr. Carl Ramsey, of Dorset, Vermont. Previous work (Curtis, 1937) has shown that one species of orchid may harbor more than one species of fungus, and it was desired further to test the extent of this phenomenon.

The following procedure of isolation was followed throughout. Seemingly healthy roots (those having no apparent sign of decay) were washed with running tap water; all adhering dirt particles were removed by careful brushing. Sample roots were examined microscopically for the presence of an endophytic fungus by means of free-hand sections. The occurrence of fungi could easily be detected by the presence of tangled knots of hyphae (Bernard's peletons) in the cortical cells and also by single hyphal strands passing from cell to cell. Roots showing such fungi were again washed, this time with sterile water, and were then externally sterilized by immersion in a 1 per cent HgCl2 solution for five minutes. They were then rinsed in sterile water and placed in a sterile Petri dish where they were cut into one centimeter sections by means of a scalpel. All operations were performed under aseptic conditions. The root pieces were distributed on potato-dextrose agar in Petri dishesabout five to one dish. The dishes were then inverted and placed in an incubator at 25° C. Examinations were made frequently and all Rhizoctonias were transferred as soon as their purity could be ascertained. All other fungi which were obviously growing from the cortical tissues at the ends of the root piece were also transferred. It may be objected that this method might allow occasional root surface contaminants to appear on the isolation plates, but in practice such contaminants were very rare, as the mercury sterilizing agent was very efficient in killing the soil organisms adhering to the root. All other methods, such as the picking of individual fungus peletons from single cells by microtechnique, are open to the same objection of the occasional presence of external organisms and, in addition, are unsuited for rapid isolation from the large numbers of roots necessary in the present investigation.

All cultures were kept on potato-dextrose agar in tubes. They were transferred to thin layers of the same agar in Petri dishes for microscopic examination of the mode of spore formation and other developmental details. Cultures were also maintained in liquid media, using such solutions as Richard's and potato-dextrose. Microscope slide mounts were made by teasing out a bit of the spore-bearing hyphae from a plate culture, staining with either cotton blue or acid fuchsin, and mounting in lactophenol. The photomicrographs were made from such slides.

ORCHID RHIZOCTONIAS.—The fungi commonly thought of as orchid symbionts have been assigned to the genus Rhizoctonia DeC. Bernard placed them in this genus because of the marked resemblance of one of them (Rhizoctonia mucoroides Bern.) to Rhizoctonia violacea Tulasne. In general the orchid Rhizoctonias have the following characteristics: mycelium composed of white, orange, reddishbrown, dark brown, or black hyphae with vertical cross walls. Hyphal branches originate near the distal end of the long cells of the main hyphae; frequent anastomosing of the branches occurs. As the mycelium ages, short inflated moniliform filaments. rich in globular inclusions, arise in the same manner as the vegetative branches. They anastomose in some species, remain free in others. The filaments are soon cut up into varying numbers of more or less inflated cells, resembling spores. Additional "spore" cells are formed either by budding from the terminal cell or by basipetal abscission. The "spore" cells may be spread uniformly throughout the mycelium or be aggregated into larger or smaller groups of closely appressed and intertwined chains of cells, here termed sporodochia. The "spore" cells vary in different species from elongate cells of only slightly greater diameter than the vegetative hyphae to swollen, spherical cells many times larger than the normal hyphae.

Bernard (1904) was the first to isolate Rhizoctonias from orchids and the first to describe them as distinct species. He named three species—Rhizoctonia repens, Rhizoctonia lanuginosa, and Rhizoctonia mucoroides. Additional species have been described by Costantin and Dufour (1920), Wolff (1926), and Burgeff (1932, 1936). Table 1 lists the described species of Rhizoctonia, with their host plants and the dates of description.

Many of the fungi isolated from Wisconsin orchids may be definitely recognized as belonging to some of the above species. Others are intermediate in character, and still others are distinctly different. The value of creating new species to cover these distinct forms is perhaps questionable, since the perfect form of none of them has been described,

Table 1. The described orchid species of Rhizoctonia.

Fungus	Host	Date of description
R. repens Bernard	Laeliocattleya Canhamiana	1909
R. lanuginosa Bernard	Odontoglossum grande	1909
R. mucoroides Bernard	Phalaenopsis amabilis	1909
R. goodyera-repentis Costantin	Goodyera repens	1920
R. neottiae Wolff	Neottia nidus-avis	1926
R. aerea Burgeff	Taeniophyllum glandulifera	1932
R. arachnion Burgeff	Thrixspermum arachnites	1932
R. floccosa Burgeff	Myrmechis glabrae	1932
R. anomala Burgeff	Orchis maculata	1936
R. asclerotica Burgeff	Ophrys apifera	1936
R. gracilis Burgeff	Oncidium sphacelatum	1936
R. robusta Burgeff	Oncidium Ĉavendishianum	1936
R. sclerotica Burgeff	Ophrys muscifera	1936
R. Stahlii Burgeff	Platanthera chlorantha	1936
R. subtilis Burgeff	Lycaste Skinneri	1936

but if the species be considered form-species, such designation may be justified on the basis of greater convenience.

The following paragraphs list the *Rhizoctonia* species isolated in the present investigation, their host plants, and a brief description of each. All descriptions of growth characters and spore sizes are based on Petri dish and test tube cultures on potato-dextrose agar and Erlenmeyer flask cultures on a liquid potato-dextrose medium. The spore sizes are given in microns and represent the average range found in fully matured spores. The photomicrographs of all species are presented at the same magnification ( $\times 208$ ).

Rhizoctonia neottiae Wolff.—Sporodochia dark red-brown, large (1 mm. in diameter), open textured. Hyphae coarse, 9–10  $\mu$  in diameter, dark brown, producing irregular appressorium-like cells on short branches when growing next to glass. Spores elongate, 74–80  $\mu$  by 19–22  $\mu$ . Figure 142, page 141, Burgeff, 1936.

Isolated from: Aplectrum hyemale, low mixed hardwoods, Jefferson County, Wisconsin.

Rhizoctonia borealis n. sp.—Mycelium at first light tan, later dark brown. Sporodochia numerous, up to 3 mm. in diameter, distinct from the rapidly growing mycelium. Mat of aerial hyphae formed in liquid cultures. Spores clostridium-like,  $37-42~\mu$  by  $24-28~\mu$ . Figure 1. Very similar to R. mucoroides Bernard except for the much greater size. The hyphal tips exhibit an unusually regular dichotomous branching prior to spore formation.

Isolated from: Goodyera repens var. ophioides, balsam-spruce swamp, Vilas County, Wisconsin; Spiranthes gracilis, sandy opening in coniferous forest, Vilas County, Wisconsin.

Rhizoctonia gracilis Burgeff.—Mycelium white. Sporodochia yellow, waxy, coalescing. Spores very elongate, only slightly greater in diameter than the vegetative hyphae,  $42-45~\mu$  by  $10-12~\mu$ . Figure 132, page 136, Burgeff, 1936.

Isolated from: *Habenaria lacera*, tamarack-sphagnum bog, Ozaukee County, Wisconsin.

Rhizoctonia robusta Burgeff.—Mycelium white. Sporodochia small, orange, waxy. Spores keg- or barrel-shaped,  $32-34~\mu$  by  $25-28~\mu$ . Figure 2.

Isolated from: *Habenaria leucophaea*, wet *Rynchospora* swale in tamarack bog, Ozaukee County, Wisconsin.

Rhizoctonia lanuginosa Bernard.—Mycelium white. Sporodochia yellowish, waxy, opalescent. Spores elongate, widest beyond the middle,  $20-23 \mu$  by  $10-16 \mu$ . Figure 3.

Isolated from: Goodyera tesselata, virgin hemlock forest, Vilas County, Wisconsin; Calopogon pulchellus, low prairie, Waukesha County, Wisconsin.

Rhizoctonia Stahlii Burgeff.—Abundant white aerial hyphae. Sporodochia yellow, large, opentextured, not waxy, mostly 1 mm. or less in diameter. Spores elongate, 26–34 μ by 14–18 μ. Figure 4.

Isolated from: Habenaria leucophaea, low prairie, Jefferson County, Wisconsin; Spiranthes cernua, moist meadow, Waukesha County, Wisconsin; Habenaria psycodes, moist meadow, Iowa County, Wisconsin.

Rhizoctonia sclerotica Burgeff.—Abundant white aerial hyphae. Sporodochia orange, compact, not waxy, at length very large, 5 mm. or more in diameter. Spores rare, except in sporodochia, 20–22  $\mu$  by 12–14  $\mu$ . Figure 5.

Isolated from: Cypripedium reginae, moist meadow, Columbia County, Wisconsin; Habenaria leucophaea, wet Rynchospora swale in tamarack bog, Ozaukee County, Wisconsin; Notylia trisepala, Oaxaca, Mexico; Spiranthes cernua, low prairie, Jefferson County, Wisconsin.

Rhizoctonia subtilis Burgeff.—Mycelium at first light tan, later becoming uniform light brown, recumbent, smooth, glistening. Sporodochia small, confluent over surface of mycelium. Spores in irregular, zig-zag, or unevenly angular chains, 8-11  $\mu$  by 5-8  $\mu$ . Figure 6.

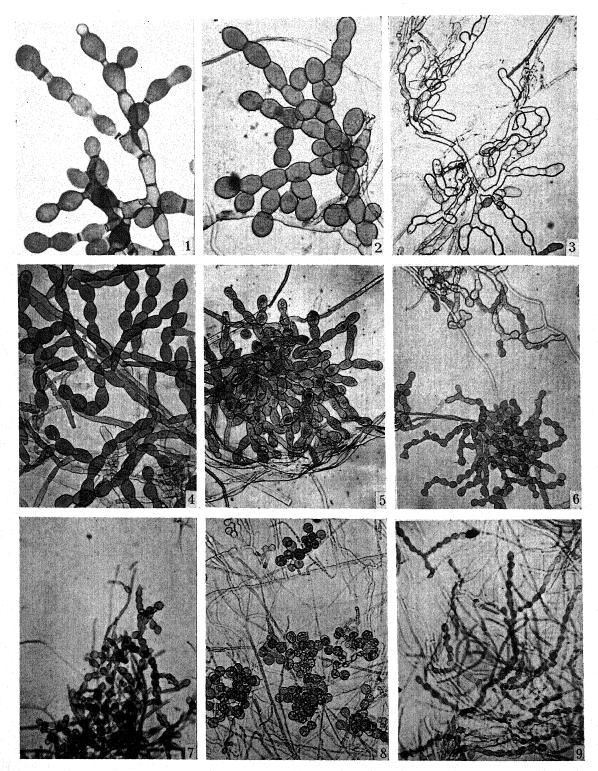


Fig. 1-9. Rhizoctonia species.—Fig. 1. R. borealis.—Fig. 2. R. robusta.—Fig. 3. R. lanuginosa.—Fig. 4. R. Stahlii.—Fig. 5. R. sclerotica.—Fig. 6. R. subtilis.—Fig. 7. R. subtilis var. nigra.—Fig. 8. R. repens.—Fig. 9. R. monilioides. All ×208.

Isolated from: Cypripedium candidum, moist meadow, Columbia County, Wisconsin; Cypripedium parviflorum, moist meadow, Columbia County, Wisconsin; Habenaria leucophaea, Rynchospora swale in tamarack bog, Ozaukee County, Wisconsin; Trichopila tortilis, Volcano San Martin, Vera Cruz, Mexico.

Rhizoctonia subtilis var. nigra n. var.—Sporodochia rare or absent. Mycelium recumbent, smooth, glistening, black, producing a black discoloration on agar. Spores infrequent, irregular and variable in shape, often constricted in the middle,  $10-13~\mu$  by  $7-9~\mu$ . Hyphae slender,  $2~\mu$  in diameter. Distinguished from the preceding by the color and by the larger size of the spores. Figure 7.

Isolated from: Spiranthes cernua, low prairie,

Jefferson County, Wisconsin.

Rhizoctonia repens Bernard.—Mycelium white, recumbent, creeping, no aerial hyphae. Sporodochia small, white, confluent in a continuous layer over the surface of agar media. Spores nearly spherical, in staphylococcoid cluster of short branched chains,  $13-19~\mu$  by  $10-14~\mu$ . Figure 8. Mycelium entirely submerged in liquid cultures.

Isolated from: Brassia giveoudiana, San Isidro, Costa Rica; Calopogon pulchellus, moist prairie, Waukesha County, Wisconsin; Goodyera pubescens, pine covered talus slope, Sauk County, Wisconsin; Goodyera repens var. ophioides, hemlock woods,

Sheboygan County, Wisconsin; Habenaria psycodes, moist quartzite ravine, Sauk County, Wisconsin; Liparis Loeselii, sphagnum bog, Ozaukee County, Wisconsin; Pogonia ophioglossoides, open sphagnum bog, Ozaukee County, Wisconsin; Spiranthes cernua, habitat unknown, Hanover, New Hampshire.

Rhizoctonia monilioides n. sp.—Cottony aerial hyphae present on agar cultures. Sporodochia absent. Spores in long (10–18 spores), branched, monilioid chains, 10–14  $\mu$  by 7–10  $\mu$ . Growth in liquid culture similar to that of R. repens. Separated from that species because of the very long spore chains, the slightly smaller spores, and the

absence of sporodochia. Figure 9.

Isolated from: Habenaria dilatata, wet Rynchospora swale in tamarack bog, Ozaukee County, Wisconsin; Habenaria hyperborea, shaded sphagnum bank, Ozaukee County, Wisconsin; Liparis liliifolia, rocky, dry, pine woods, Sauk County, Wisconsin; Pogonia ophioglossoides, wet Rynchospora swale in tamarack bog, Ozaukee County, Wisconsin; Sobralia pleiantha, San Isidro, Costa Rica; Spiranthes Romanzoffiana, tamarack-sphagnum bog, Vilas County, Wisconsin.

It will be noted from the isolation data of the preceding fungi that most of them were found in more than one orchid species. More important, however, is the observation that the same orchid spe-

Table 2. Orchid species with the Rhizoctonia species isolated from them.

					Fur	ngi				
Orchids	R. borealis	R. gracilis	R. lanuginosa	R. monilioides	R. neottiae	R. repens	R. robusta	R. sclerotica	R. Stahlii	R. subtilis
Cypripedieae		-								
Cypripedium candidum			٠,٠,							X
C. parviflorum	٠.									×
C. reginae			• . • .				• • •	$\times$	• •	٠, ٠,٠
Ophrydeae										
Habenaria dilatata				×		• •				
H. hyperborea				X						٠
H. lacera		$\times$	· · · ·		• •		٠.			٠,
H. leucophaea							$\times$	×	$\times$	$\times$
H. psycodes		• •		٠. ٠		$\times$	٠		×	
Neottieae										
Pogonia ophioglossoides				×	٠	×	• •			
Calopogon pulchellus			×			X	٠	٠		
Spiranthes cernua	٠			٠,	٠.	$\times$	٠	$\times$	×	, ×
S. gracilis	×		• •							٠, ٠.
S. Romanzoffiana				×	•					•
Goodyera pubescens					• • •	×			٠.,	
G. repens var. ophioides	×	• •			••	×	•••		• •	
G. tesselata	•••		$\times$			• •	• •		• •	•
Epidendreae										
Liparis liliifolia				×	•.•					×
L. Loeselii						×	• ,•			
Aplectrum hyemale		٠			×		٠.			

cies may harbor more than one species of Rhizoctonia. In one instance R. robusta and R. subtilis, two very different species, were isolated from the same root piece of Habenaria leucophaea (Curtis, 1937). Table 2 lists the species of orchids and the

species of fungi isolated from them.

Both Habenaria leucophaea and Spiranthes cernua harbored four distinct fungus species, and it seems quite probable that the number of species found in the other orchids was limited only by the lack of a sufficient number of isolations. This is particularly true in the Goodyeras, where only a few plants were available. In Cypripedium, a great many isolations were attempted, not only from C. candidum, C. parviflorum, and C. reginae, but also from C. acaule, C. arietinum, and C. pubescens. Only three Rhizoctonias were obtained in more than twenty-five trials. In most of the attempts, the excessive amount of raphides and other crystals in the root cells apparently prevented the growth of the fungi present or had caused their death while still within the living root.

From table 2 it will be seen that there is no apparent specificity about the infection of orchids by Rhizoctonia species. Any one orchid may be infected by several species of fungi, and the same fungus may infect widely separated species of orchid. Even when grouped into the larger unit of the tribe, the orchids show no tendency to be infected by any specific fungus or group of fungi. The only statement that can be made is that orchids on the whole are subject to attack by various species of fungi, with the genus Rhizoctonia furnishing the largest number and the most common parasites of

the group.

Previous work (Curtis, 1937) has indicated that there might be a correlation between fungus species and the habitat of the host orchid. Additional proof of this contention was found in the present investigation. Thus, Rhizoctonia sclerotica was obtained from orchids growing in a low prairie, in a moist open meadow, and in a Rynchospora swale in a tamarack bog. All these locations were similar in that they were fairly moist, were exposed to the full sun, and had a soil reaction between pH 7.2 and 7.6. Again, R. Stahlii was isolated from three species of orchids growing in essentially the same conditions as the hosts of R. sclerotica—an open meadow, and two low prairies, with the same conditions of moisture, light, and soil reaction. Burgeff (1936) states that R. Stahlii is found in orchids growing in damp situations-"Meist Pilze von Orchideen feuchterer Standorte." He isolated it from Platanthera, Ophrys, and Coeloglossum, all typical orchids of the alkaline European chalk meadows.

Another example is Rhizoctonia borealis, found only in the acid soil areas of the Canadian Zone coniferous forest. R. subtilis and its variety nigra occurred only in moist, open, alkaline habitats—a moist meadow, a low prairie, and an alkaline swale in a tamarack swamp.

On the other hand, Rhizoctonia lanuginosa, R. repens, and R. monilioides show no such limitations as to ecological requirements. The former occurred in an acid forest and an alkaline meadow in Wisconsin and has been isolated from several epiphytes of the American tropics. R. repens has been isolated from orchids growing in a great range of habitats in Wisconsin. Other investigators have isolated it from a number of tropical orchids of widely different ecological distributions. However, since most of these isolations were made from greenhouse or conservatory grown specimens, nothing definite can be said about the natural range of R. repens. In this connection it is of interest to point out that of the eleven isolations of this species made by Bernard, eight came from one greenhouse, two from another, and one from a third. He also found R. mucoroides in Vanda and Phalaenopsis from one greenhouse and found it in the fern Ophioglossum in the same

With most species the correlation between type of habitat and the species of fungus found in orchids growing in that habitat seems quite distinct. For example, in Wisconsin, any moist, open, alkaline meadow or swale would be expected to harbor Rhizoctonia sclerotica, R. Stahlii, and R. subtilis. Any orchid growing in such a habitat might be infected by any one or more of these forms, with no specific relationship between the species of orchid and the species of fungus. All three, for instance, have been found in Habenaria leucophaea.

All the evidence from these isolations points to the conclusion that the various Rhizoctonia species are soil saprophytes of either widespread or more restricted distribution, and that they may become facultative parasites, attacking the roots of any orchids in the immediate vicinity. These isolation results do not, by themselves, show that there is no symbiotic relation between orchid and fungus. It might be argued that, although more than one fungus can enter the roots of an orchid, only one is of benefit in seed germination. It will be remembered that the ability to induce seed germination is the main proof of a true mutual symbiosis offered by

the proponents of the symbiotic theory.

In order to test this possibility, a series of experiments was conducted in which all the fungi isolated from an orchid were planted in symbiotic cultures with the seeds of that orchid. Fungi isolated from other orchids were also planted with the same seeds. Burgeff's medium Sb (1936) at pH 5.2—the test solution recommended for determining the germinating power of fungi-was used as a substrate throughout. The controls were planted on the same medium except that the carbohydrate source was glucose, rather than starch, as orchid seeds cannot be expected to grow in non-symbiotic culture when provided with an insoluble carbohydrate. The results are shown in table 3. Although rather limited in number, these results tend to show that the fungi isolated from an orchid are not necessarily capable of germinating its seeds. In the

Table 3. Results of symbiotic germination tests, 0 indicates no germination. × indicates good germination, and the underline indicates those trials in which the fungus used was isolated from the species bearing the seeds.

				F	ungi				
Orch'd species—seeds	R. borealis	R. gracilis	R. lanuginosa	R. monilioides	R. repens	R. sclerotica	R. Stahlii	R. subtilis	Control
Cypripedium parviflorum			×	0	0			0	×
Cypripedium reginae	0			$\times$	$\times$	0		0	. 0
Habenaria leucophaea		0	•			$\overline{0}$	0	0	0
Calopogon pulchellus			0		0	٠	$\bar{\times}$		×
Goodyera pubescens				×	×				X
Liparis Loeselii		0		0	.0			٠	0
Laeliocattleya Fascinator		×	$^{\prime}$ $\times$	×	$\bar{\times}$	0	×	0	×
Vanda itangiana		×	0	0	$\overline{\times}$			$\times$	×

case of Habenaria leucophaea and Liparis Loeselii, where no germination occurred in any instance, the results might be interpreted as being due to poor seeds, but since the seeds were freshly collected and apparently viable, the results are probably valid.

Numerous other examples of a similar nature are to be found in the literature. Bernard (1904) was unable to germinate the seeds of Spiranthes autumnalis with Rhizoctonia repens isolated from that orchid. He also noted that fungi isolated from the same Cattleya plant at different times of the year varied greatly in their ability to induce germination of Cattleya seeds. Burgeff (1909) was unable to germinate the seeds of seventeen European terrestrial orchids (including three genera and two species of North American distribution) with their own fungi or with fungi of other temperate orchids.

In other experiments with terrestrial orchids of Java, Burgeff (1936) found that the seeds of eleven species of eight genera failed to germinate when planted with fungi isolated from them or isolated from other species of the same genera. He also found that Rhizoctonia mucoroides isolated from Phalaenopsis equestris would bring about the germination of seeds of Vanda and Euanthe, but not those of Phalaenopsis schilleriana, P. Aphrodite, nor P. stuartiana.

Ramsbottom (1927) also noted instances of the occurrence of fungi in orchid roots that would not induce germination of that orchid's seeds. He says, "Several isolations of the Cattleya fungus were made. These fungi varied to a great extent, and with the first two or three sets of them no great success in germination was attained. . . After a short time a strain was isolated which gave a totally different picture for Cattleya. Here germination occurred in a most astonishing way . . . one could almost guarantee over ninety per cent germination."

According to Ames (1938), J. N. Porter found that the seeds of Zeuxine strateumatica would not

germinate when planted with a species of *Rhizoctonia* isolated from the roots of that orchid. This is especially interesting, since *Zeuxine* is an introduced species that has been spreading throughout the State of Florida in recent years and apparently germinates readily under field conditions.

A similar line of evidence is presented by the numerous instances of orchid seeds induced to germinate by fungi other than those isolated from the roots of the seed-bearing species. Thus Cypripedium reginae would not germinate with Rhizoctonia sclerotica from that orchid, but would germinate with R. repens from Pogonia ophioglossoides and with R. monilioides from Liparis liliifolia. Again, Bernard (1909) reported germination of Brassolaelia with R. mucoroides from Phalaenopsis and of Vanda with R. lanuginosa from Odontoglossum. He also reported that R. mucoroides induced better germination of Cymbidium seeds than did R. repens isolated from Cymbidium. Burgeff (1936) pointed out that R. repens isolated from the North American terrestrial orchid Habenaria psycodes was able to induce germination of seeds of Angraecum from Madagascar, of a large number of tropical epiphytes from Central and South America, and of both epiphytic and terrestrial orchids from the Indomalayan mountains.

Discussion.—A review of these results indicates that the roots of orchids are easily infected by various saprophytic fungi, among which various species of Rhizoctonia are especially common. The Rhizoctonia species vary widely in their ecological requirements and probably also in their geographical distribution. Those growing in any particular location are apt to infect any orchids growing in that same location. If the orchids are capable of growing in several types of habitat, they are open to a greater variety of infection than if they are restricted to a single habitat. In any case they may be infected by several species of Rhizoctonia, since more than one fungus species is found in most loca-

tions and in addition at least one species (R. repens) is apparently almost universal in distribution.

When seeds of any orchid are planted on an essentially unfavorable medium, such as one containing starch or other insoluble carbohydrate supply, germination may be induced by an appropriate fungus inoculated on to the culture. This fungus may or may not be a member of the genus Rhizoctonia. If not, it may be a basidiomycete (Pleurotus, Corticium, Hypholoma, Marasmius), an ascomycete (Ceratostomella, Penicillium, Chaetomium), or a phycomycete (Phytophthora, Choanophora). If a Rhizoctonia, it need not be one which grows in the roots of that orchid or in closely related orchids. Indeed, the fungi from many orchids will not induce the germination of the seeds of those orchids and may be definitely injurious to them.

When the freshly collected seeds of a great many orchids are planted non-symbiotically on a favorable medium, such as one containing a mineral nutrient solution of low osmotic concentration, a soluble carbohydrate supply, and nitrogen in the form of some complex proteinaceous compound, germination and subsequent development proceed in a normal fashion. In the case of old seeds of certain orchids (i.e., Cymbidiinae and Sarcanthinae) the addition of certain growth substances may be necessary to bring about successful germination.

The evidence offered here showing that there is no specific relation between fungus species and orchid host, and the demonstration by other investigators that orchid seeds may germinate without the aid of any fungus or with fungi unrelated to *Rhizoctonia*, both point to the conclusion that the mycorrhizal association is devoid of mutualistic significance.

#### SUMMARY

The arguments in favor of the existence of a mutually beneficial symbiosis between orchids and fungi are considered to be successfully refuted by the work of Knudson and others, except for the hypothesis of specificity of the orchid-fungus relationship. To test this theory, isolations of mycorrhizal fungi were made from 23 species of orchids from various habitats in the United States and Mexico. Ten species of Rhizoctonia were obtained. including two new species, Rhizoctonia borealis, n. sp., and R. monilioides, n. sp. No evidence of specificity could be found, as one orchid species could harbor as many as four distinct fungus species, and one fungus species could attack at least seven different orchids of four distinct tribes. There appeared to be a correlation of fungus species with ecological habitat, rather than with orchid species. A few fungi were found which occurred in a wide variety of habitats.

Symbiotic germination tests were made, using all the fungi isolated from a given orchid in combination with the seeds of that orchid. The results supported the above contention of non-specificity, since the fungi isolated from an orchid were in most cases unable to induce germination of that orchid's seeds. In view of both Knudson's demonstration that the fungus is unnecessary for germination and the evidence offered here that the orchid-fungus relation is non-specific, it is concluded that the symbiotic relationship is one of parasite and host, with the orchid deriving no benefits from the fungus in its roots.

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# THE ABSORPTION OF PHOSPHATE AND NITRATE BY ILLUMINATED CULTURES OF NITZSCHIA CLOSTERIUM $^{\rm 1}$

## Bostwick H. Ketchum

The researches of Brandt (1899, 1902) first called attention to the importance of phosphate and nitrate as factors limiting phytoplankton growth in the sea. Numerous investigations have since been made using accurate analytical methods, and the results show that during the growth of the phytoplankton, phosphate and nitrate may be removed completely from the water (Atkins, 1923, 1926a, 1926b; Cooper, 1933; Harvey, 1926, 1928; Harvey et al., 1935; Kreps and Verjbinskaya, 1932; Marshall and Orr, 1928). Some results indicate that the growth of phytoplankton ceases when these ions have completely disappeared from the water (Marshall and Orr, 1928).

The conclusions reached by study of the variables in the sea have been corroborated by laboratory experiments. Schreiber (1928) determined the total number of diatoms which could be grown in water collected at various times of the year and found a good correlation between the concentration of phosphate and the crop. Braarud and Foyn (1931) showed that nitrate can also limit diatom growth.

Although the concentrations of phosphate and nitrate in sea water vary between wide limits, Redfield (1934) showed that they vary in a related fashion. The ratio of concentrations in the waters of the West Atlantic, South Atlantic, Pacific, and Indian Oceans and Barents Sea is about one atom of phosphorus to twenty atoms of nitrate nitrogen. Redfield also shows that the proportions of carbon, nitrogen, and phosphorus in typical hawls of various plankton are nearly the same as the proportions found in the sea water. He pointed out that these relations do not obtain in water bodies of limited extent, such as the English Channel, the Gulf of Maine, etc., where local conditions may alter the picture. Cooper (1937) discusses the departure from the usual ratio in the English Channel and in the Mediterranean Sea but offers no explanation for these anomalies.

Redfield (1934) states: "Whatever its explanation, the correspondence between the quantities of biologically available nitrogen and phosphorus in the sea and the proportions in which they are utilized by the plankton is a phenomenon of the greatest interest."

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The absorption of phosphate and nitrate by pure cultures of a marine diatom has been studied to determine what effect its growth might have upon the ratio of these ions in the water. This paper presents data relating the absorption of phosphate and nitrate by cells grown under continuous illumination to the concentration of these ions in the medium. In those ranges of concentration normally found in the sea the rate of absorption per cell is dependent on the concentrations of the nutrient in the water. The rate of phosphate absorption is dependent on both the nitrate and the phosphate concentration, while the rate of nitrate absorption is dependent on the concentration of nitrate in the medium and independent of the phosphate concentration.

As a consequence of this relationship the ratio in which phosphate and nitrate are absorbed is dependent on the concentration of phosphate in the medium and independent of the nitrate concentration over large ranges. The growth of the cells changes the proportions in which phosphate and nitrate are present in the medium. Under certain conditions the metabolism of the cells brings the ratio of phosphorus to nitrogen closer to the usual value found in the sea; under other conditions it increases an original departure from this ratio.

Material and methods.—The marine diatom, Nitzschia Closterium, which was used in this work was grown in the medium suggested by Ketchum and Redfield (1938). Stock cultures in 500 cc. Erlenmeyer flasks were prepared regularly and were grown in the light of a north window. These cultures were seeded with a concentration of 50,000 cells per cc. and contained, at the end of five or six days, about two million cells per cc. At this time they were used as seed for the new stock and for the experimental work. Tests for bacteria were made regularly by plating on the medium described by Reuszer (1933), as well as by the methods suggested by Barker (1935a). All the experiments described were performed on bacteria-free cultures.

The phosphate in the medium was measured by the Deniges-Atkins ammonium molybdate method, using stannous chloride as the reducing agent (Atkins, 1923). Since the cells present in the medium give the solution a greenish color which will not match with the standards, the cultures were filtered through a barium sulphate precipitate on a cintered glass filter, as suggested by Redfield, Smith, and Ketchum (1937). Salt effect and blank corrections were always made (cf. Redfield et al.).

The nitrate nitrogen in the medium was measured by the reduced strychnine method described by Harvey (1926) for use in sea water. The modifications introduced by Rakestraw, as discussed by Wattenburg (1937), were used throughout. The color was allowed to develop for eight hours and was compared in a bicolorimeter with standards consisting of methyl orange in 0.001 normal HCl. Concentrations ranging from 5 to 100 gamma (grams ×10<sup>-6</sup>) of nitrogen per liter can be measured directly. Higher concentrations must be measured by dilution with "nitrate free" sea water. Corrections were made for the amount of nitrate added in the diluting water. The reagent measures both nitrate and nitrite nitrogen. The latter is always present in relatively low concentrations, and nitrate was used for enrichment of the culture medium. The measurements were recorded, therefore, as nitrate. All figures given in the paper are the average of two or more check analyses.

The experimental cultures were grown in a specially constructed air chamber. An electric refrigerating unit is set into one-half of the top of an insulated box. The other half is occupied by a window consisting of two sheets of glass, separated by one and one-half inches of insulating air space. The inner sheet is finely ground in order to diffuse the light. The light source consists of four 100-watt lamps mounted on the corners of a twenty-inch square of plywood, the center of which is occupied by a 75-watt bulb. The bulbs are an inch above the outer glass plate. The cultures are supported by a shelf within the chamber ten inches below the glass window. This lighting arrangement gives uniform illumination on the shelf which is more than adequate to produce maximal growth in the cultures when the cell concentration is 50,000 cells per cc. The temperature is controlled by a mercury thermoregulator, the air being circulated by an electric fan.

EXPERIMENTAL RESULTS.—The absorption of nitrate.—In order to determine the effect of the concentration of phosphate in the medium on the rate of absorption of nitrate, experiments were performed in the following way: cells from a stock culture which had been grown in the light of a north window for six days were placed in fresh sea water,

and the cell concentration was adjusted to 50,000 cells per cc. The suspension was enriched with nitrate and then divided in flasks. Different amounts of phosphate were added to each flask. The cultures were placed in the dark for twelve hours and were then placed in the light at a temperature of 16°C. The rate of absorption was determined by the difference between the nitrate present when the cultures were placed in the light and the final concentration of nitrate in the medium. Any differences in the rate of absorption of nitrate under these conditions may be attributed to the different phosphate concentrations, since all other variables are controlled.

The results of three experiments performed in this way at different nitrate concentrations are presented in table 1. In each experiment the phosphate concentration varies between less than ten and about one hundred gamma per liter. The data show that this tenfold increase in the concentration of phosphate has no influence on the rate of absorption of nitrate from the medium.

Since these experiments were performed at three different nitrate concentrations they show the effect of the concentration of nitrate on the rate of its absorption. The cells suspended in the medium containing about 240 gamma of nitrate nitrogen per liter absorbed two and one-half times as much nitrogen as those in the medium containing only fifty gamma per liter.

In order to study in more detail the relation between the concentration of nitrate in the medium and the rate of absorption of nitrate, experiments were performed in which the nitrate concentration was different in the various cultures. Although the phosphate concentration in these cultures was not equal, the data presented in table 1 show that this does not affect the rate of nitrate absorption. The results are summarized in table 2, where they are arranged according to the original concentration of nitrate in the medium. The rate of absorption increases steadily with the increase in the concentration of nitrate in the medium. In figure 1 the relation between the average concentration of nitrate in the medium and the rate of absorption of nitrate by the cells is shown. The data from tables 1 and 2

Table 1. The absorption of nitrate by illuminated cultures of Nitzschia Closter um at 16°C.

The original cell concentration was adjusted in all cases to 50,000 cells per cc.

Original phosphate gamma/L.	Original nitrate gamma N/L.	Final nitrate gamma N/L.	Nitrate absorbed gamma N/L.	Time hours	Nitrate absorbed per cell per hour gamma
<10	47	5	42	12	7.0, 10-8
94	50	7	43	12	7.2 "
<10	147	70	77	12	12.8 "
15	108	72	36	5.8	12.4 "
22	150	114	36	5.8	12.4 "
105	127	46	81	12	13.5 "
<10	247	133	114	12	19.0 "
94	236	137	99	12	16.5 "

Table 2. The absorption of nitrate by illuminated cultures of Nitzschia Closterium at 16°C. in relation to the concentration of nitrate in the medium. The cell concentration was adjusted in all cases to 50,000 cells per cc.

r	riginal nitrate nme N/L.	Final nitrate gamma N/L.	Average nitrate gamma N/L.	Nitrate absorbed gamma N/L.	Time hours	Nitrate absorbed per cell per hour gamma
	63	32	47	31	5.8	10.7, 10-8
	97	24	60	73	12.0	12.1 "
	100	62	81	38	5.8	13.1 "
	128	38	83	90	12.0	14.9 "
	183	108	145	75	11.5	13.0 "
	187	94	140	93	12.0	15.5 "
	200	112	156	88	12.0	14.6 "
	270	165	217	105	12.0	17.5 "
	300	241	270	59	5.8	20.3 "
	319	219	269	100	12.0	16.7 "
	326	241	284	85	10.5	16.2 "

are included in this figure. Although the experimental variation is large at the high concentrations of nitrate, since these must be measured by dilution, the increasing rate of absorption with increasing concentration is plainly demonstrated. Above a concentration of about 200 gamma nitrate nitrogen per liter the rate of absorption of nitrate appears to approach a limiting value.

These experiments show that although the rate of absorption of nitrate is independent of the concentration of phosphate, it is directly related to the concentration of nitrate in the medium.

The absorption of phosphate.—In the utilization of phosphate by diatoms two types of absorption can be distinguished. Absorption from the medium always occurs when the cells are grown in the light, provided phosphate is available. Phosphate is also absorbed in the dark by cells which are deficient in phosphorus, as described in another paper (Ketchum, 1939). Since this effect is so pronounced, it is necessary to be certain that it is not confusing the results obtained in the study of the absorption

by illuminated cultures. It was found that stock cultures should be no more than five or six days old when used. Cells from such a culture are resuspended in fresh sea water and placed in the dark for a period of twelve or more hours. By this time the "phosphate debt" is satisfied. The cultures are then placed in the light, and the absorption of phosphate from the medium may be measured.

Experiments were performed on cells prepared in this way to determine the influence of the phosphate concentration in the medium on the rate of absorption of phosphate. The cultures were divided in flasks which were placed in the light at 16°C. after the addition of a different amount of phosphate to each flask. The phosphate concentration was measured when they were placed in the light, and the rate of absorption was determined by the difference between this and the final concentration of phosphate in each case.

The results of three experiments performed in this way at three different nitrate concentrations are presented in table 3 and figure 2. In each ex-

Table 3. The rate of absorption of phosphate by illuminated cultures of Nitzschia Closterium at 16°C. in relation to the concentration of phosphate in the medium at three different nitrate concentrations.

Original nitrate gamma N/L	Original phosphate gamma/L.	phosphate		Time hours	Initial P count cells/L.	hosphate absorbed per cell per hour gamma
0-5	45	32	13	6	230, 106	0.94, 10-8
0-5	70	52	18	6	"	1.30 "
0-5	104	82	22	6	· · · · · · · · · · · · · · · · · · ·	1.60 "
0-5	188	163	25	6	<b>«</b>	1.81 "
65	92	45	47	13.5	60, 106	5.8, 10-8
65	132	77	55	13.5	46	6.8 "
69	167	104	63	13.5	"	7.8 "
63	264	199	65	13.5	<b>"</b>	8.0 "
220	11	6	5	6	50, 106	1.7, 10-8
220	43	19	24	6	"	8.0 "
220	94	64	30	6	<b>"</b>	10.0 "
220	253	220	33	6	"	11.0 "

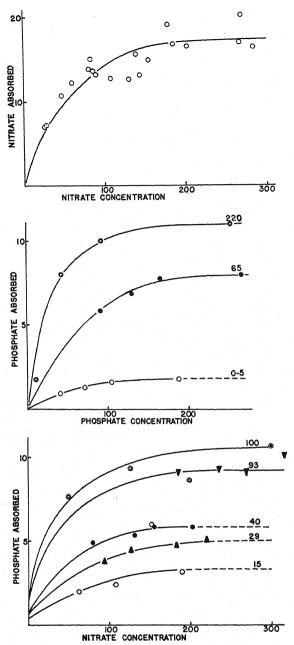


Fig. 1-3.—Fig. 1. The relation between the rate of absorption of nitrate by illuminated cultures of Nitzschia Closterium and the concentration of nitrate in the medium. Abscissa, concentration of nitrate in the medium. Abscissa, concentration of nitrate absorbed per cell per hour, units gamma  $N\times10^8$ .—Fig. 2. Relation between the rate of absorption of phosphate by illuminated cultures of Nitzschia Closterium at 16°C. and the concentration of phosphate in the medium, at three different concentrations of nitrate. The figure at the end of each curve shows the nitrate concentration for that experiment; units—gamma N per liter. Ordinate, phosphate absorbed per cell per hour, units gamma  $PO_4\times10^8$ . Abscissa, concentration of phosphate in the medium, units gamma  $PO_4$  per liter.—Fig. 3. Relation between

periment the rate of absorption of phosphate increases with an increase in the phosphate concentration in the medium. The rate of absorption of phosphate also depends on the concentration of nitrate in the medium, as comparison of the results obtained at the three different nitrate concentrations shows.

The experiment in which the cultures contained no nitrate is particularly interesting. Since nitratefree sea water is difficult to obtain, a culture was allowed to grow until no nitrate could be detected in the medium by the reduced strychnine method. It was then divided in four flasks and a different amount of phosphate added to each flask. After six hours in the light a definite absorption of phosphate was measured, showing a dependence of the rate of absorption on the phosphate concentration which is similar to that demonstrated by the other two experiments. From this experiment it is clear that the cells are able to absorb phosphate from the medium, even when no measurable nitrate is present. Comparison with the other two experiments, however, shows that the presence of nitrate materially increases the rate of absorption of phosphate.

Experiments were also performed to study the effect of the concentration of nitrate on the rate of absorption of phosphate from the medium. In each of these experiments the original concentration of phosphate was equal, whereas the concentration of nitrate was different in the various flasks. The amount of phosphate absorbed by the cells was determined by the difference between the initial concentration of phosphate and the concentration after a period of illumination at 16°C. The results of five experiments performed in this way are presented in table 4 and figure 3, where the values plotted for the zero concentration of nitrate are taken from the experiment reported in figure 2. Again a family of curves is obtained which shows the dependence of the rate of absorption of phosphate on the concentrations of both phosphate and nitrate. Each curve of the family was obtained for a different concentration of phosphate, which is given by the figure at the end of the curve. All the curves show a relation between the rate of absorption of phosphate and the nitrate concentration. Intercomparison of the curves shows that, for a given concentration of nitrate, the rate of absorption of phosphate increases with increasing concentrations of phosphate.

The rate of growth under the experimental conditions used.—It was possible that the various experimental conditions used might influence the rate

the rate of absorption of phosphate by illuminated cultures of Nitzschia Closterium at 16°C. and the concentration of nitrate in the medium at five different phosphate concentrations. The figure at the end of each curve shows the phosphate concentration at which that experiment was performed, units gamma per liter. Ordinate, phosphate absorbed per cell per hour, units gamma  $PO_4 \times 10^8$ . Abscissa, concentration of nitrate in the medium, units gamma N per liter.

Table 4. The rate of absorption of phosphate by illuminated cultures of Nitzschia Closterium at 16°C. in relation to the concentration of nitrate in the medium at various phosphate concentrations.

Original nitrate gamma N/L.		Final phosphate gamma/L.	Phosphate absorbed gamma/L.	Time hours	Initial count cells/L.	Phosphate absorbed per cell per hour gamma
63	15	10	5	5	50, 106	2.0, 10-8
108	15	9	6	5	"	2.4 "
154	15	0	15	5	. "	6.0 "
190	15	7	8	5	"	3.2 "
94	29	8	21	11.5	50, 106	3.7, 10-8
128	29	4	25	11.5	"	4.4 "
183	29	2	27	11.5		4.8 "
222	29	0	29	11.5	••	5.0 "
80	40	29	11	4.5	50, 106	4.9, 10-8
131	40	28	12	4.5	46	5.3 "
156	40	27	13	4.5	***	5.8 "
204	40	27	13	4.5	•	5.8 "
186	93	38	55	12.0	50, 106	9.2, 10-8
236	93	37	56	12.0	"	9.4 "
270	93	38	55	12.0	••	9.2 "
319	93	32	61	12.0	•	10.2 "
50	100	45	55	12.0	60, 106	7.7, 10-8
127	100	32	68	12.0	"	9.4 "
202	100	38	62	12.0	"	8.7 "
300	100	68	32	5.0	<b>"</b>	10.6 "

of growth of the cells and thus indirectly control the rate of absorption of phosphate and nitrate. Experiments were therefore performed to discover what effect the concentrations of nitrate and phosphate in the medium have upon the rate of growth. These experiments were done in the same way as those reported above, except that they were grown in the light of a north window instead of in the artificially illuminated air bath. Longer periods of time were used in these experiments in order to obtain significant differences in the cell counts. A di-

rect measure of the rate of growth is given by the division rate per cell (k), which is defined by the equation  $k=1/t\left[\ln C_{\rm t}/C_{\rm o}\right]$  in which  $C_{\rm t}$  and  $C_{\rm o}$  are the cell counts at the end and beginning of time t.

The results of the experiments are summarized in table 5. In the first experiment the effect of the nitrate concentration on the rate of growth was studied. All these cultures were started with an equal phosphate concentration, and the nitrate concentration varied from 47 to 525 gamma per liter.

Table 5. The growth of cultures of Nitzschia Closterium in media containing various nitrate and phosphate concentrations. Grown in the light of a north window at room temperature.

Expt.	Initial phosphate	Initial nitrate	Initial count	Final count	Time	Division rate (k)
no.	gamma/L.	gamma N/L.	cells/L.	cells/L.	hours	Days-1
1	50	47	46.0, 106	170, 106	45	0.70
	50	185	47.0 "	170 "	45	0.68
	50	375	49.0 "	180 "	45	0.69
	50	525	44.0 "	170 "	45	0.72
2	15	500	16.7 "	26.7 "	36	0.31
	30	500	16.7 "	38.8 "	36	0.56
	50	500	16.7 "	45.1 "	36	0.67
	70	500	16.7 "	47.6 "	36	0.70
	110	500	16.7 "	45.6 "	36	0.68
3	54	500	44.4 "	170 "	45	0.72
	105	500	46.0 "	160 "	45	0.67
	145	500	47.0 "	170 "	45	0.69
4	52	150	46.0 "	180 "	48	0.68
-	103	150	50.0 "	180 "	48	0.64
	153	150	47.0 "	190 "	48	0.70

All the experiments on the absorption of nitrate from the medium were performed within this range of concentrations. The rate of growth in all these flasks was nearly constant in spite of this tenfold increase in the concentration of nitrate.

The second, third, and fourth experiments reported in table 5 relate the rate of growth with the concentration of phosphate in the medium. They show that between 50 and 150 gamma of phosphate per liter the rate of growth is independent of the phosphate concentration. Below a concentration of 50 gamma of phosphate per liter, however, the rate of growth is limited by the concentration of phosphate in the medium.

Since the rate of growth is constant at different concentrations of phosphate and nitrate, the differences in the rate at which these ions are absorbed is not due to different growth rates. Concentrations of phosphate below 50 gamma per liter, however, may limit the rate of absorption of phosphate indirectly by affecting the rate of growth. It is interesting to note that such low concentrations of phosphate have no influence on the rate of absorption of nitrate (see table 1). It appears, therefore, that in these experiments the effect of the rate of growth is negligible in comparison with the direct effect of the concentration of the nutrients on the rate of absorption.

Table 6. The proportions in which phosphate and nitrate are absorbed from media containing various concentrations of these ions as predicted from data presented in tables 1, 2, 3, and 4 and in figures 1, 2, and 3.

Phosphate conc. Gamma/L.	Nitrate conc. gamma N/L	$ ext{PO}_4$ abs. $ ext{cell/hour}$ . $ ext{gamma}{ imes}10^8$	NO <sub>3</sub> abs. /cell/hour gamma×108	Conc. ratio PO <sub>4</sub> :N grams:grams	Abs. ratio $\Delta PO_4:\Delta N$ grams:grams	Abs. ratio ΔP:ΔN atoms:atom
15	10	0.7	3.1	1.5	0.23	0.034
15	20	1.0	5.4	0.75	0.18	0.027
15	50	1.6	9.4	0.30	0.17	0.025
15	100	2.4	13.8	0.15	0.17	0.025
15	200	3.2	17.0	0.075	0.19	0.028
15	300	3.2	17.2	0.05	0.19	0.028
30	10	1.2	3.1	3.0	0.39	0.057
30	20	1.6	5.4	1.5	0.30	0.044
30	50	2.8	9.4	0.6	0.30	0.044
30	100	4.0	13.8	0.3	0.29	0.043
30	200	5.0	17.0	0.15	0.29	0.043
30	300	5.1	17.2	0.10	0.30	0.044
40	10	1.7	3.1	4.0	0.55	0.081
40	20	2.2	5.4	2.0	0.41	0.060
40	50	3.6	9.4	0.8	0.38	0.056
40	100	5.1	13.8	0.4	0.37	0.055
40	200	5.9	17.0	0.2	0.35	0.052
40	300	5.9	17.2	0.13	0.34	0.050
65	10	1.8	3.1	6.5	0.58	0.085
65	20	2.6	5.4	3.25	0.48	0.083
65	50	4.2	9.4	1.30	0.45	0.066
65	100	6.3	13.8	0.65	0.45	0.066
65	200	7.5	17.0	0.33	0.44	0.065
65	300	7.5	17.2	0.22	0.44	0.065
90	10	3.0	3.1	9.0	0.97	0.143
90	20	4.6	5.4	4.5	0.85	0.143
90	<b>5</b> 0	5.8	9.4	1.8	0.62	0.124
90	100	7.8	13.8	0.9	0.57	0.091
90	200	9.4	17.0	0.45	0.55	0.081
90	300	9.4	17.2	0.3	0.55	0.083
200	10	3.2				
200	20	3.2 4.9	3.1	20.0	1.03	0.152
200	50 50	6.6	5.4	10.0	0.91	0.134
200	100	9.1	9.4	4.0	0.70	0.103
200	200		13.8	2.0	0.66	0.097
200	300	10.0 10.1	17.0	1.0	0.59	0.087
			17.2	0.65	0.59	0.087
300	10	3.5	3.1	30.0	1.13	0.167
300	20	5.0	5.4	15.0	0.92	0.135
300	50	6.8	9.4	6.0	0.72	0.106
300	100	9.2	13.8	3.0	0.67	0.099
300	200	11.0	17.0	1.5	0.65	0.096
300	300	11.0	17.2	1.0	0.64	0.095

Since the rate of absorption of phosphate and nitrate was measured on illuminated cultures, cell division must have taken place. The growth rates recorded in table 5 indicate that the cells would almost double in number in the eight to ten hours of continuous illumination allowed for these experiments. The rates of absorption have been expressed in terms of the initial number of cells in the culture. No correction for the amount of growth has been made. Expressed in this way the results are comparable for all experiments except those containing less than 50 gamma of phosphate per liter, where lower rates of growth are observed.

Ratio of absorption.—From the data concerning the rate of absorption of phosphate and nitrate from media of various compositions it is possible to predict the ratio in which these ions will be absorbed from media in which they occur in any proportion. The ratio in which phosphate and nitrate are absorbed at seven phosphate and six nitrate concentrations have been calculated, using the rates of absorption obtained from figures 1, 2, and 3. These are presented in table 6, where they are grouped according to the phosphate concentration, each group containing the data for six nitrate concentrations. The ratio of absorption,  $\Delta P : \Delta N$ , is constant in each group, even though the nitrate concentration varies between 10 and 300. It will be remembered that the rate of absorption of both nitrate and phosphate are dependent upon the nitrate concentration. The effect of the nitrate upon the absorption of these two ions must be similar or the constant ratio would not be obtained. This similarity is not found at low nitrate concentrations, since these limit the rate of absorption of nitrate to a greater extent than the rate of absorption of phosphate. An increase in the value for the ratio of absorption is observed for these low nitrate concen-

Intercomparison of the groups shows that the value for the ratio increases with increasing concentrations of phosphate.

In the fifth column of the table the ratio in which phosphate and nitrate are present in the medium is shown. For each phosphate concentration these vary thirtyfold, since the nitrate concentration varies between 10 and 300. Comparison of these figures with the ratio of absorption shows that the latter is completely independent of the proportions of nitrate to phosphate in the medium.

Under normal conditions of growth both phosphate and nitrate are being continuously removed from the medium. Since the concentration of phosphate in the medium determines the ratio in which these ions are absorbed, this ratio must constantly change while the cells are growing. In figure 4 the changes in the medium during constant growth of the cells are presented. The light lines show the changes in the medium predicted from the data in table 6. Simultaneous measurements of the phosphate and nitrate remaining in the medium after various periods of illumination were made on a num-

ber of cultures. These data are included in figure 4, where the points represent the composition of the medium at various times. The heavy lines connecting these points show how the growth of the

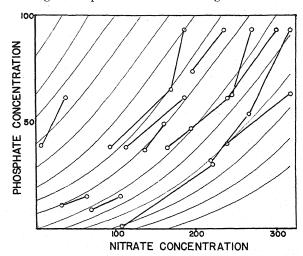


Fig. 4. The simultaneous removal of phosphate and nitrate from sea water of various compositions. The light lines show the changes predicted in table 6 from the data on the rate of absorption. The points, connected with heavy lines, show the changes observed when simultaneous measurements of phosphate and nitrate concentrations are made on actively growing cultures. Ordinate concentration of nitrate in the medium, units gamma N per liter. Abscissa concentration of phosphate in the medium, units gamma  $PO_4$  per liter.

cells changes the composition of the medium. The changes observed in these cultures agree closely with the predicted changes in the medium.

The usual ratio of phosphate phosphorus to nitrate nitrogen in the sea would be represented in figure 4 by a line drawn from the origin through the medium containing 298 gamma of nitrate nitrogen and 100 gamma of phosphate (PO<sub>4</sub>). It is apparent that this line does not coincide with the way in which phosphate and nitrate are absorbed by the cells. If the ratio P:N in the medium is high, the metabolism of the cells brings the ratio closer to the usual one; if the ratio is low, the metabolism of the cells increases the original departure from the usual ratio.

Discussion.—Schreiber (1928) and Braarud and Foyn (1931) found that the growth of unicellular algae is limited by low concentrations of phosphate and nitrate, respectively. In their experiments the concentrations of the nutrients studied was low, and they were undoubtedly exhausted before the end of the experiments. Such experiments certainly show that a deficiency of an essential element reduces the total crop which can be produced in a given medium, but no conclusions can be drawn concerning the rate of growth at a given concentration. Barker (1935b) showed that the rate of growth of marine dinoflagellates is inde-

pendent of the concentration of nitrate in the medium. The concentrations used in his work were sufficiently high so that they were not exhausted. The data presented in this paper confirm Barker's conclusions, since the rate of growth of Nitzschia Closterium is independent of the concentration of nitrate and phosphate over the concentration range of natural sea water.

It has been commonly assumed that low concentrations of a nutrient in natural waters limit the rate of growth and the rate of absorption of the nutrient simultaneously. The data presented in this paper show that this is not always true. The rate of absorption of both phosphate and nitrate varies in media containing different concentrations of these ions, even though the rate of growth is independent of their concentrations.

The data concerning the proportions in which nitrate and phosphate are absorbed indicate that at least two synthetic processes involving phosphorus are taking place and that these reactions are rigorously controlled by the concentration of both phosphate and nitrate in the medium. One of these reactions apparently involves both nitrogen and phosphorus metabolism and is controlled by the concentration of nitrate in the medium. It will be remembered that regardless of the concentration of nitrate in the medium the proportion in which phosphate and nitrate are absorbed is constant for a given phosphate concentration. Since the different nitrate concentrations influence the rate of absorption of both phosphate and nitrate, these rates must increase in direct proportion, as though a compound containing both nitrogen and phosphorus were being synthesized.

Phosphorus must also be utilized in reactions which do not involve nitrogen. The rate at which these reactions proceed must be dependent on the concentration of phosphate in the medium, since increased concentrations of phosphate increase the

rate of absorption of phosphate with no influence on the rate of absorption of nitrate.

The various reactions involving phosphorus can be most readily studied in unicellular forms, since the rates at which they proceed can be so easily controlled. This study is being extended to the formation of various organic compounds of phosphorus under the conditions discussed in this paper.

#### SUMMARY

The rate of absorption of nitrate by the marine diatom, Nitzschia Closterium, is independent of the concentration of phosphate in the medium, but it increases as the concentration of nitrate in the medium increases.

The rate of absorption of phosphate is directly dependent on the concentration of both phosphate and nitrate in the medium.

The rate of growth is independent of the concentrations of phosphate and nitrate in the medium except for phosphate concentrations of less than 50 gamma per liter, when it decreases.

The ratio in which phosphate and nitrate are absorbed is constant for each concentration of phosphate in the medium, regardless of the concentration of nitrate, except that at very low concentrations of nitrate the ratio of absorption increases.

The ratio of absorption  $\Delta P : \Delta \tilde{N}$  increases as the concentration of phosphate in the medium increases.

The normal course of absorption in cultures does not directly correspond to the usual ratio found in sea water. If the cells are in a medium in which the ratio of concentrations is somewhat higher than usual, the absorption by the cells tends to reduce the ratio toward the usual value. If the ratio of concentrations is lower than usual, the metabolism tends to increase the discrepancy.

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## STUDIES OF SOUTH AMERICAN PLANTS. VIII. NEW AND NOTEWORTHY SPECIES OF LECYTHIDACEAE <sup>1</sup>

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No comprehensive monograph of the Lecythidaceae has been published other than that of Miers, since which an abundance of herbarium material has been collected in tropical America. Many species of the family have been described since 1874, but no keys to species in the large genera, such as Eschweilera, have been published, with the exception of those in Eyma's excellent treatment3 of the Surinam species. In Amazonian Brazil and the Guianas alone, more than 80 names have been referred to Eschweilera. Although some of these will doubtless fall into synonymy, there certainly remain many species yet to be collected, and one may anticipate that the genus will eventually include at least 150 species in its entire range. As most members of the family are large trees with very hard wood, they have been neglected by most collectors until recently.

The following notes are based upon material in the herbarium of the New York Botanical Garden, in which types of the new species are deposited.

GUSTAVIA PLEUROCARPA Pittier. Colombia: Bolivar: Puerto Canoa to Salvajin, on Rio Esmeralda, Pennell 4548. Santander: Vicinity of Puerto Wilches, on Rio Magdalena, Killip & Smith 14904. The species has previously been reported only from the vicinity of the Panama Canal Zone.

GUSTAVIA RHODANTHA Standl. Colombia: Bolivar: Tierra Alta, on Rio Sinu, *Pennell 4629*. Like the preceding, this species appears to be new to South America, having been otherwise reported only from the San Blas District of Panama.

Eschweilera montana sp. nov. Arbor ad 25 m. alta; ramulis subteretibus juventute striatis fuscis cinereo-puberulis mox glabris et cinereis; foliis glabris, petiolis rugosis superne leviter angulatis 5-6 mm. longis, laminis coriaceis utrinque laevibus siccitate olivaceo-fuscis oblongo-ellipticis, 8-14 cm.

1 Received for publication April 22, 1939.

<sup>2</sup> MIERS, J. 1874. On the Lecythidaceae. Trans. Linn. Soc. 30: 157-318. Pl. 33-65.

<sup>3</sup> EYMA, P. J. 1934. Lecythidaceae. Pulle, Fl. Surinam 3: 119-155.

longis, 3-5.5 cm. latis, basi acutis et petiolo leviter decurrentibus, apice breviter et obtuse acuminatis, margine leviter revolutis et subintegris vel obscure crenulatis, costa utrinque prominente, nervis secundariis utroque 6-9 arcuato-adscendentibus inconspicue anastomosantibus subtus planis et inconspicue immersis subtus leviter elevatis, venulis reticulatis utrinque planis vel obscuris; inflorescentiis terminalibus vel axillaribus paniculatis pauciramosis 3-7 cm. longis praeter rhachidem ramulos et pedicellos molliter cinereo-puberulos glabris; rhachide gracili leviter flexuosa; pedicellis gracilibus 6-10 mm. longis, bracteis bracteolisque mox deciduis; calvce cupuliformi, limbo suberecto, lobis 6 anguste imbricatis oblongo-ovatis 2-3 mm. longis et latis, apice rotundatis; petalis 6 eburneis submembranaceis tenuiter nervatis inaequalibus oblongo-obovatis, sub anthesi 11-14 mm. longis et 6-10 mm. latis, apice rotundatis; androphoro explanato circiter 20 mm. longo, ligula circiter 6 mm. lata, galea 9-12 mm. diametro spiraliter incurvata appendiculis anantheris 3-4 mm. longis dense echinata; staminibus numerosis (circiter 150), filamentis 1-1.3 mm. longis, antheris subglobosis circiter 0.5 mm. diametro; ovario semisupero vertice valde convexo umbonato laevi glabro, loculis 2, ovulis in quoque locula 6-10, stylo anguste conico circiter 1 mm. longo.

Type, Smith 2990, collected January 19, 1938, in dense forest of the Akarai Mountains, on height of land between drainage of Rio Mapuera (Trombetas tributary) and Shodikar Creek (Essequibo tributary), Brazil-British Guiana Boundary, alt. 600-800 m. E. montana belongs to a group of small-flowered species including E. micrantha (Berg) Miers, E. grata Sandwith, and E. floribunda Eyma. From all of these it is readily distinguished by its smooth leaves with more obscure secondaries and veinlets, and from the last, perhaps its closest ally, by its less freely branched inflorescence, glabrous eciliate sepals, and slightly larger petals.

Eschweilera alata sp. nov. Arbor 20–28 m. alta ubique glabra, trunco 15–35 cm. diametro; ramulis

fuscis gracilibus conspicue bialatis (alis subcoriaceis 1-1.5 mm. latis); petiolis crassis rugosis leviter canaliculatis 5-15 mm. longis; laminis coriaceis siccitate fusco-olivaceis oblongo-ellipticis, 15-27 cm. longis, 5-10 cm. latis, basi rotundatis vel obtusis vel subattenuatis et petiolo decurrentibus, apice gradatim breviter acuminatis, margine subintegris vel obsolete crenatis vel conspicue undulatis, costa utrinque prominente, nervis secundariis utroque 9-12 patentibus vel subadscendentibus marginem versus inconspicue anastomosantibus utrinque elevatis, rete venularum crasse intricato utrinque conspicue prominulo; inflorescentiis terminalibus (et axillaribus?) simplicibus 10-15 cm. longis, rhachide gracili (internodiis 1-2 mm. diametro) leviter flexuosa striata 10-15-flora, pedunculo brevi; pedicellis erassis rugosis 8-20 mm. longis distaliter incrassatis, 1-3 mm. supra basin conspicue articulatis, cum calyce (ex coll.) viridibus vel purpurascentibus; calycis tubo turbinato rugoso, lobis 6 patentibus ovatooblongis, 4-5.5 mm. longis et latis, apice rotundatis, margine saepe ciliolatis; petalis 6 eburneis vel basi pallide roseo-purpurascentibus, obovatis, 20-25 mm. longis, 15-25 mm. latis, apice rotundatis, margine fimbriatis; androphoro eburneo explanato 35-40 mm. longo, ligula subcoriacea 12-14 mm. lata, galea 15-20 mm. diametro spiraliter incurvata, parte apicali extus appendiculis 3-5 mm. longis dense echinata; staminibus numerosis, filamentis clavatis 1.3-2.5 mm. longis, apice mucronulatis, antheris oblongis circiter 0.7 mm. longis; ovario subinfero vertice leviter elevato umbonato, loculis 2 multiovulatis, stylo brevi crasso.

Type, British Guiana Forestry Dept. 2564 (D541), collected November 11, 1937, in Morabukea mixed forest near Kumuparu Creek, basin of Demerara River, British Guiana. Another collection from the same locality is Forestry Dept. 2560 (D537). These specimens were kindly sent to me for study by Prof. S. J. Record, and duplicates are deposited in the Yale School of Forestry. The native name is reported as "Guava-skin Kakaralli," in contrast to "Smooth-leaved Kakaralli" (E. decolorans Sandwith), which occurs in the same region.

E. alata is distinguished by its large glossy leaves with coarsely reticulate venation, its simple lax comparatively few-flowered inflorescences, its large flowers with white petals, its coiled androecium, and its 2-celled ovary. It is also characterized by having its branchlets, at least distally, narrowly 2-winged; this character separates it from the allied E. subglandulosa (Steud.) Miers, as do also the coarseness of the veinlet reticulation, the usually longer pedicels, and the larger flowers.

Eschweilera rhododendrifolia (Knuth) comb. nov. Chytroma rhododendrifolia Knuth in Fedde Rep. Sp. Nov. 38: 116. 1935. Krukoff 7991, from essentially the type locality near Manaos, Amazonas, Brazil, is referred to this species with some misgivings, the type not being available to me at present. Compared with the description, our speci-

men is somewhat more robust throughout, with leaf blades proportionately slightly narrower and inflorescences longer. These characters are hardly of specific significance, but the present plant has the ovary 2-celled, whereas Knuth's reference of his plant to *Chytroma* implies a 4-celled ovary, although this is not stated in the description.

Eschweilera conduplicata sp. nov. Arbor ad 20 m. alta ubique glabra; ramulis crassis rugosis demum cinereis inconspicue lenticellatis; petiolis crassis canaliculatis 9-17 mm. longis; laminis coriaceis siccitate olivaceo-viridibus late ellipticis vel leviter oblongo-obovatis, 9-15 cm. longis, 4.5-8 cm. latis, basi rotundatis vel obtusis vel subattenuatis et saepe petiolo decurrentibus, apice rotundato-obtusis vel obtuse et breviter acuminatis, margine leviter recurvatis et subintegris vel obsolete crenatis, inter costam et margines sulculis utroque 2-4 curvatis plerumque signatis, costa supra elevata subtus prominentissima et striata, nervis secundariis utroque 14-18 patulis inconspicue anastomosantibus supra subplanis vel obtuse elevatis subtus prominulis, rete venularum arcte intricato supra plano vel minute prominulo subtus manifeste prominulo; inflorescentiis axillaribus et terminalibus simplicibus vel raro basin versus pauciramosis 11-30 cm. longis, rhachide crassa flexuosa angulata 20-50-flora; pedicellis crassis valde rugosis 1-3 mm. longis persistentibus, basi bracteatis, apice bracteolatis et conspicue articulatis (bracteis bracteolisque caducis non visis); floribus in articulo sessilibus expansis circiter 4 cm. diametro; calyce purpureo, tubo valde verruculoso, lobis 6 margines versus virescentibus coriaceis patulis anguste imbricatis oblongis, 6-8 mm. longis et latis, apice rotundatis; petalis 6 roseis tenuiter carnosis oblongis, 16-20 mm. longis, 11-16 mm. latis, apice rotundatis, margine integris; androphoro carnoso explanato 35-40 mm. longo, ligula 10-15 mm. lata, galea rosea supra ovarium inflexa neque spiraliter incurvata 14-17 mm. diametro, parte inflexa intus appendiculis incrassatis linearibus apicem versus directis echinata; staminibus numerosissimis (300 vel ultra), filamentis complanatis 2.5-4 mm. longis, apice abrupte contractis et filiformibus, antheris luteis subglobosis 0.5-0.7 mm. diametro; ovario subinfero vertice leviter convexo striato-sulcato sub anthesi 3-4 mm. diametro, loculis 4, ovulis in quoque loculo 10-14; stylo carnoso 2-3 mm. longo, stigmate minute 4-lobato, lobis reflexis.

Type, Smith 2332, collected November 1, 1937, in forest in basin of Rupununi River, near mouth of Charwair Creek, latitude about 2°35'N., British Guiana. It is fairly abundant locally along the forest fringes in the upper Rupununi region, apparently occurring not along the river itself, but on the inner edges of the forest near the sharp line of transition to the savanna which covers the major portion of the region. Search in other parts of the Rupununi region did not disclose the species in a second locality. It is a striking and beautiful tree, conspicuous for its rich pink petals and androecia.

E. conduplicata is related to a small group of predominantly Amazonian species with coriaceous leaves, a simple or few-branched long inflorescence with a stout angled flexuose rachis, short-pedicellate or essentially sessile flowers of medium size for the genus, an androecium not coiled inwards but simply bent over the ovary, and a 4-loculed ovary. Among its relatives are  $E.\ rorida$  (Miers) A. C. Smith, E. retusa (Spruce) Nied., and E. ovalifolia (DC.) Nied. From these the new species is distinguished by the conspicuously finer reticulation of its veinlets, by the prominent marks of conduplicate vernation of its leaf blades (perhaps not a stable character but very pronounced in available material), by the larger sepals, and by other details of flower size and texture.

Eschweilera alutacea sp. nov. Arbor ad 25 m. alta ubique glabra; ramulis nigrescentibus vel cinereis crassis rugosis dense lenticellatis; petiolis crassis profunde canaliculatis 4-8 mm. longis; laminis coriaceis siccitate supra fusco-olivaceis subtus brunneis late ellipticis, 12-19 cm. longis, 7-9.5 cm. latis, basi rotundatis vel leviter subcordatis et petiolo abrupte decurrentibus, apice breviter acuminatis, margine subplanis et obsolete crenatis, costa utrinque prominente et striata, nervis secundariis utroque 15-17 patentibus marginem versus arcuatis et inconspicue anatomosantibus utrinque acute elevatis, rete venularum intricato supra leviter prominulo fere plano subtus conspicuo; inflorescentiis axillaribus et terminalibus simplicibus vel basin versus binis 12-19 cm. longis, rhachide crassa (internodiis 2-3 mm. diametro) angulata conspicue lenticellata 25-40-flora, pedunculo subnullo; pedicellis crassis (circiter 3 mm. diametro) 1-2 mm. longis apice conspicue articulatis bracteolatis (bracteolis crasse coriaceis) persistentibus; floribus in articulo sessilibus expansis 5-6 cm. diametro; calycis tubo valde rugoso sub anthesi 3-4 mm. longo et 7-9 mm. diametro inconspicue lenticellato, lobis 6 coriaceis ovato-oblongis, 10-12 mm. longis, 8-10 mm. latis, apice rotundatis, margine saepe obscure glandulosis; petalis 6 albidis tenuiter carnosis oblongo-obovatis, 25-30 mm. longis, 13-23 mm. latis, apice rotundatis, margine saepe glanduloso-ciliolatis; androphoro albido distaliter flavido explanato 50-55 mm. longo, ligula 11-15 mm. lata, galea supra ovarium inflexa neque spiraliter incurvata 20-25 mm. diametro, parte inflexa valde incrassata intus appendiculis ad 10 mm. longis patentibus dense echinata; staminibus numerosissimis (200 vel ultra), filamentis clavatis 3-4 mm. longis, apice mucronulatis, antheris luteis oblongo-subglobosis circiter 0.6 mm. diametro; ovario subinfero vertice plano vel leviter convexo plurisulcato, loculis 4, ovulis in quoque loculo 10-12; stylo carnoso cylindricoconico 4-5 mm. longo, stigmate minute 4-lobato.

Type, Smith 2690, collected December 17, 1937, in dense forest along Essequibo River, near mouth of Onoro Creek, latitude about 1°35'N., British Guiana. E. alutacea is characterized by broad thick

short-petioled leaves, long stout inflorescences, large subsessile flowers with white petals and a yellowish androecium, a 4-loculed ovary, and an androecium simply bent over the ovary but not coiled inwards. It belongs in the same general alliance as the preceding (E. conduplicata) and its allies, but is more robust throughout and differs in flower color, apparently a dependable character in the genus. A nearer relationship is with E. holcogyne Sandwith, from which it differs by its substantially larger leaves with more numerous secondaries, much longer and more robust inflorescence, broader sepals and petals, and longer style. Perhaps a closer ally, according to the description, is Eschweilera Miersii (Knuth) A. C. Smith. comb. nov. (Chytroma Miersii Knuth in Fedde, Rep. Sp. Nov. 38: 114. 1935), from which E. alutacea differs by its short petiole, thicker leaves with more spreading secondaries, shorter and much slenderer rachis, and much smaller calyx tube.

Eschweilera confertiflora sp. nov. Arbor ad 31 m. alta, rhachidibus, pedicellis, et saepe basin versus calycibus cinereo-puberulis exceptis glabra, trunco circiter 30 cm. diametro; ramulis teretibus cinereis gracilibus; petiolis nigrescentibus striatis canaliculatis superne anguste alatis 3-6 mm. longis; laminis tenuiter coriaceis siccitate fuscis oblongoellipticis 5.5-8 cm. longis, 2-3.5 cm. latis, basi acutis, apice breviter cuspidatis et calloso-apiculatis, margine subintegris vel obsolete crenatis, costa utrinque valde elevata, nervis secundariis utroque 11-13 rectis patentibus marginem versus anastomosantibus cum rete venularum arcte intricato utrinque valde prominulis; inflorescentiis axillaribus et terminalibus simplicibus 8-13 cm. longis, rhachide crassa (internodiis circiter 2 mm. diametro) angulata lenticellata 45-55-flora, pedunculo subnullo; pedicellis 3-5 mm. longis 1-3 mm. supra basin conspicue articulatis, bractearum cicatricibus conspicuis, bracteolis caducis; pedicellis apicem versus et calveis tubo valde verruculosis; sepalis 6 oblongoovatis, circiter 3 mm. longis et 2 mm. latis, apice rotundatis; petalis 6 submembranaceis oblongo-obovatis, 8-12 mm. longis, 6-8 mm. latis, apice rotundatis; androphoro explanato circiter 15 mm. longo, ligula circiter 5 mm. lata basi expansa, galea supra ovarium inflexa neque spiraliter incurvata 7-9 mm. diametro, parte inflexa intus appendiculis ad 2 mm. longis deorsum (basin versus) directis dense echinata; staminibus numerosis, filamentis ligulatis vel clavatis 1.5-3.5 mm. longis, apice mucronulatis, antheris subgloboso-oblongis 0.4-0.5 mm. diametro; ovario subinfero vertice leviter elevato et obscure plurisulcato, loculis 4, ovulis in quoque loculo 3-5; stylo subulato carnoso circiter 2.5 mm. longo; pyxidio immaturo valde lenticellato turbinato, vitta interzonali suberecta, sepalis persistentibus, operculo leviter convexo conspicue apiculato.

Type, British Guiana Forestry Dept. 2557, collected November 5, 1937, in climax forest along Kumuparu Creek, basin of Demerara River, British

Guiana. The specimen was kindly forwarded to me for study by Prof. S. J. Record, and a duplicate is in the herbarium of the Yale School of Forestry (serial no. 35448). Unfortunately the flower color is not recorded, but in dried condition the petals have a pinkish tinge. E. confertiflora is distinguished by an unusual combination of characters; it has small coriaceous leaves with densely reticulate and strongly prominulous veinlets, long stout angled simple puberulent many-flowered inflorescences, short pedicels conspicuously articulate near the middle, comparatively small flowers, an androecium bent over the ovary but not spirally incurved, and a 4-celled ovary. It appears to be without close relatives, but may be compared, among Guiana species, to E. corrugata (Poit.) Miers, E. chartacea (Berg) Eyma, E. Wachenheimii (Ben.) Sandwith, and E. praeclara Sandwith, from all of which it is distinguished by its leaf size and venation and its inflorescence characters.

BERTHOLLETIA EXCELSA H. B. K. British Guiana: basin of Shodikar Creek (Essequibo tributary), latitude about 1°18'N., Smith 2868. The species is fairly abundant in this locality, where it was in good flowering condition in January and also bearing maturing fruits of the previous year. It is said to be a dominant tree farther north near King William's Falls on the middle Essequibo and also in the upper Corentyn region.

COURATARI TENUICARPA A. C. Smith. Since this species was originally described on the basis of a fruiting specimen from the basin of Rio Madeira, it has been collected in good flowering condition (Ducke 497, from Rio Taruma, near Manaos, Amazonas, Brazil). The following notes should be considered in connection with the original description:

Inflorescentiae ubique (i.e. rhachide, pedicellis, bracteis, calveibus, sepalis, et petalis extus) dense cinereo- vel fusco-puberulae; racemis e ramulis efoliatis ut videtur saepe orientibus simplicibus 7-15 cm. longis; rhachide gracili subtereti 10-20flora; pedicellis gracilibus 11-22 mm. longis, bracteis papyraceis oblongis 3-4 mm. longis subtentis; sepalis 6 anguste imbricatis semiorbiculari-ovatis, 1.5-2 mm. longis, 2-3 mm. latis, apice rotundatis, margine fimbriatis; petalis 6 membranaceis glabrescentibus tenuiter nervatis oblongo-obovatis, 17-22 mm. longis, 7-11 mm. latis, basi angustatis, apice rotundatis, margine integris et fimbriatis; androphoro glabro explanato 17-20 mm. longo, ligula 5-7 mm. lata, galea 6-8 mm. diametro laevi; staminibus circa annulum circiter 20, filamentis gracilibus 0.3-0.4 mm. longis, antheris transversaliter ovoideis circiter 0.5 mm. longis et 0.7 mm. latis; ovario turbinato vertice leviter convexo et glabro umbonato; stylo brevi crasso; loculis 3, ovulis 15-20 in quoque loculo.

Couratari reticulata sp. nov. Arbor sub fructu ubique glabra 35 m. alta, trunco 80 cm. diametro; ramis ramulisque teretibus purpurascentibus juventute striatis; petiolis canaliculatis anguste alatis

7-12 mm. longis; laminis subcoriaceis ellipticis siccitate fusco-olivaceis, 11-15 cm. longis, 5-8 cm. latis, basi obtusis et petiolo abrupte decurrentibus, apice subacutis vel breviter obtuse acuminatis, margine inconspicue undulato-crenatis, costa utrinque prominente, nervis secundariis utroque 16-22 utrinque elevatis 5-10 mm. intra margines conspicue anastomosantibus, venulis copise et conspicue reticulatis utrinque valde prominulis; pyxidio obconicocylindrico vix trigonoideo 7-10 cm. longo zona calveari prominente 4-5 cm. diametro cincto, vitta interzonali erecta vel paullo convexa 5-10 mm. alta; operculo laevi circiter 3.5 cm. diametro, columella acute triquetra; pericarpio brunneo coriaceo circiter 1 mm. crasso extra laevi et dense lenticellato intus septorum 3 vestigiis signato; seminibus in quoque loculo circiter 4 elliptico-obovatis, circiter 5 cm. longis et 2.2-2.8 cm. latis, scuto embryonifero centrali ad 3.5 cm. longo et 1 cm. lato ala membranacea cincto.

Type, Smith 3561, collected April 13, 1938, in dense forest on northwestern slopes of Kanuku Mountains, in drainage of Moku-moku Creek (Takutu tributary), altitude 200 m., British Guiana. It is a tree with a straight trunk and small inconspicuous buttresses. Flowers of the new species are lacking, and unfortunately only one attached fruit containing seeds could be found, although the felled tree was thoroughly searched. Several old fruits were obtained from the ground. On foliage and fruit characters, C. reticulata can be readily distinguished, being characterized by hard elliptic leaves with crenulate margins and closely reticulate venation, firm smooth fruits of medium size for the genus, and broadly elliptic seeds. It appears to be not closely related to other Guiana species, but on the basis of its fruits to suggest C. glabra Camb. of southern Brazil. However, the leaves of that species are oblong-lanceolate, smaller, and much narrower than those of the new species, while the pyxidium is slightly different in proportions, with a higher interzonal band.

Couratari stellata sp. nov. Arbor excelsa praeter inflorescentiam ubique glabra ad 30 m. alta, trunco 50 cm. diametro; ramis ramulisque teretibus juventute purpurascentibus striatis conspicue lenticellatis mox cinereis; petiolis subcomplanatis alatis 5-10 mm. longis alis inclusis 3 mm. latis; laminis tenuiter coriaceis ellipticis, 6-10 cm. longis, 3-5.5 cm. latis, basi attenuatis petiolo decurrentibus, apice breviter (2-4 mm.) cuspidatis, margine crenatoserratis (serrationibus 5 vel 6 per centimetrum), sulculis curvatis longitudinalibus inconspicue signatis, costa supra elevata subtus prominente, nervis secundariis utroque 13-16 utrinque valde prominulis 5-7 mm. intra margines anastomosantibus, venulis copiose et conspicue reticulatis utrinque prominulis; racemis axillaribus et terminalibus simplicbus 10-15 cm. longis utrinque (i.e. rhachide, pedicellis, calycibus, sepalis, et petalis extus) dense et arcte fusco-stellato-tomentellis (pilis 6-12-ramosis sessilibus circiter 0.2 mm. longis); rhachide angulata 12-20-flora; pedicellis 4-7 mm. longis, basin versus bracteolarum cicatricibus 2 conspicuis signatis: sepalis 6 imbricatis semiorbiculari-ovatis, 4-5 mm. longis, 6-7 mm. latis, basi auriculatis. apice rotundatis vel mucronulatis, margine saepe fimbriatis; petalis 6 siccitate papyraceis valde concavis obovatis, 20-25 mm. longis, 18-22 mm. latis, intus glabris, obscure nervatis, basi angustatis, apice rotundatis, margine minute serrulatis vel erosis; androphoro glabro explanato circiter 30 mm. longo, ligula circiter 10 mm. lata, galea 8-12 mm. diametro utringue dense echinata, appendiculis lineari-subulatis ciliatis; staminibus circa annulum 40-45, filamentis 0.5-0.8 mm. longis, antheris oblongis circiter 1 mm. longis; ovario rugoso turbinato vertice plano et pubescente; stylo crasso circiter 1 mm. longo; loculis 3, ovulis 30-35 in quoque loculo; pyxidio cylindrico-conico saepe falcato, 5-6 cm. longo, zona calvcari 2.2-3 cm. diametro leviter elevata, vitta interzonali erecta 3-6 mm. alta; operculo laevi 2-2.5 cm. diametro centro abrupte depresso, columella acute triquetra; pericarpio laevi circiter 1 mm. crasso extra dense lenticellato; seminibus compressis oblongo-obovatis, scuto embryonifero centrali ala membranacea lata cincto.

Type, Krukoff 8893, collected October-December, 1936, on terra firma in high forest in basin of Creek Belem, Municipality São Paulo de Olivença, basin of Rio Solimoes, Amazonas, Brazil. C. stellata is quite distinct, on the basis of foliage and inflorescence, from any described species. It is characterized by winged petioles, finely reticulate leaf blades with crenate-serrate margins, simple racemose inflorescences, and dense close stellate pubescence of flower parts. In inflorescence characters it shows a relationship with C. macrosperma A. C. Smith, but that species, as well as the closely allied C. Krukovii A. C. Smith, is much more robust in all its parts. The pyxidium of the new species suggests that of C. paraensis Mart., otherwise unknown. It seems advisable to describe the present species as new rather than to tie it to such a poorly defined name, especially since the two plants are from very different localities.

Couratari oligantha sp. nov. Arbor; ramulis gracilibus teretibus juventute fuscis minute puberulis mox cinereis glabris; petiolis gracilibus striatis decidue puberulis superne anguste alatis 5-8 mm. longis; laminis papyraceis praeter costam supra interdum inconspicue puberulam glabris, oblongoellipticis, 7-11 cm. longis, 2-4.5 cm. latis, basi angustatis et petiolo decurrentibus, apice gradatim acuminatis (acumine ad 1 cm. longo subacuto), margine subintegris, costa supra acute elevata subtus prominente, nervis secundariis utroque 7-10 arcuato-adscendentibus marginem versus anastomosantibus cum rete venularum intricato utrinque prominulis; racemis terminalibus et axillaribus 3-6floris ubique (rhachide, bracteis minutis caducis, pedicellis, calveibus, et petalis) arcte et dense cinereo-puberulis; rhachide gracilibus ad 2.5 cm. longis; pedicellis gracilibus curvatis 15-25 mm. longis prope basin articulatis; calycis tubo sub anthesi cupuliformi circiter 3 mm. longo et 4–5 mm. diametro, sepalis 6 patentibus anguste imbricatis deltoideo-ovatis, 5–6 mm. longis, 4–5 mm. latis, apice obtusis vel rotundatis, margine ciliolatis, intus parce puberulis; petalis 6 submembranaceis venuste nervatis intus glabris, oblongis vel anguste obovatis, 20–30 mm. longis, 7–12 mm. latis, apice rotundatis, margine ciliolatis et distaliter erosis; androphoro glabro explanato 20–25 mm. longo, basi late expanso, ligula 6–8 mm. lata, galea circiter 8 mm. diametro non echinata; staminibus circiter 50, filamentis gracilibus circiter 0.5 mm. longis, antheris subglobosis circiter 0.7 mm. diametro; ovario vertice leviter elevato umbonato, stylo brevi crasso.

Type, Louis Weiss & Hermann Schmidt 1, collected in 1907 or 1908 in the region of the upper Rio Negro, Amazonas, Brazil. The local name "Tauary" is recorded; it is unfortunate that no detailed locality or habitat data are available. The slender puberulent few-flowered inflorescences with long pedicels and large flowers readily separate C. oligantha from the others of the genus. The leaves are also extremely distinct, being smaller than those of most species, narrow, and gradually taper-

ing at both ends.

Cariniana pachyantha sp. nov. Arbor excelsa ubique glabra ad 37 m. alta, trunco circiter 50 cm. diametro; ramulis crassis nigrescentibus teretibus parce lenticellatis; petiolis crassis (circiter 3 mm. diametro) canaliculatis superne anguste alatis 5-8 mm. longis; laminis tenuiter coriaceis vel papyraceis siccitate olivaceo-viridibus ovato-oblongis, 9-13 cm. longis, 5-9 cm. latis, basi rotundatis et subito petiolo decurrentibus, apice abrupte acuminatis (acumine gracili 7-12 mm. longo), margine integris et anguste revolutis, costa supra leviter elevata vel subplana subtus prominente, nervis secundariis utroque 13-19 rectis patulis 3-10 mm. distantibus marginem versus abrupte adscendentibus utrinque leviter elevatis, venulis subimmersis creberrime transversis; inflorescentiis axillaribus vel terminalibus paniculatis 6-12 cm. longis multifloris, ramulis numerosis angulato-striatis transverse rugosis; floribus sessilibus; calvcis tubo turbinato sub anthesi 1-2 mm. longo et 2-3 mm. diametro, limbo erectopatente, lobis 5-7 crasse carnosis deltoideis circiter 1 mm. longis et 1.5 mm. latis apice obtusis; petalis 5-7 crasse carnosis valde imbricatis concavis integris oblongo-obovatis, 5-6 mm. longis, 2-3.5 mm. latis, apice rotundatis; androphoro breviter tubuloso crasse carnoso oblique truncato 2.5-3 mm. alto in laciniis antheriferis abeunte; staminibus 17-25, filamentis valde reflexis 0.5-0.8 mm. longis distaliter abrupte contractis, antheris transversaliter ellipsoideis circiter 0.4 mm. longis et 0.6 mm. latis; ovario vertice plano vel leviter convexo, loculis 3, ovulis in quoque loculo 10-12; stylo carnoso circiter 0.4 mm. longo truncato.

Type, Krukoff 8690, collected October-December, 1936, in high forest on terra firma in basin of Creek Belem, Municipality São Paulo de Olivença,

basin of Rio Solimoes, Amazonas, Brazil. C. pachyantha is clearly related to C. uaupensis (Spruce) Miers, being hardly distinguishable on inflorescence characters. The new species is characterized by its much shorter petioles and differently shaped leaf blades, which are more definitely truncate at base and nearly as broad as those of C. uaupensis, although hardly half as long. The older species has 22-27 pairs of secondary nerves. C. multiflora Ducke (represented in the present collection by Krukoff 8164, 8508, and 8624 from essentially the

type locality) is more distantly of this relationship. Cariniana Micrantha Ducke. This species, originally described in 1930 and known from several localities in the basins of the Amazon, Tapajoz, Madeira, Jurua, and Purus, is now represented from the Solimoes by Krukoff 8796. C. clavata Novik. (Acta Inst. Bot. Acad. Sci. URSS I. 2: 250. f. 2. 1936) from Amazonian Colombia, described and figured from fruit only, is certainly the same species

NEW YORK BOTANICAL GARDEN

#### SEX INTERGRADES IN DIOECIOUS MAIZE 1

Donald F. Jones

THE UNISEXUAL maize plants, described previously (Jones, 1934), have been continued five additional generations. These later investigations bring out clearly the fact that the floral expression varies in different families and that this variation is under germinal control. The original dioecious condition was established by combining two recessive characters known as silkless (sk) and tassel seed  $(ts_2)$ in such a way that sk sk ts2 ts2 constituted the female plants and sk sk Ts2 ts2 the male plants. Thus the females are homozygous and the males heterozygous for the Ts2 ts2 locus on chromosome 1. It was also shown that other modifying factors influenced the floral expression such that some female plants showed varying numbers of staminate parts and that some male plants showed an occasional pistillate floret.

The general conclusion from this preliminary study was stated as follows: "One specific gene holds the balance of control over the sex of the offspring. In its action this gene is wholly dependent upon the support of at least one other gene located in a different chromosome. Other genes working with physiological modifiers, both internal and external, influence the development of the sex organs and bring about varying degrees of intergradation between the two sexes. None of these factors has been specifically located, but definite evidence for their existence is at hand. They are complex in their action and widely distributed in the chromosomes." The present report is concerned with some of these internal and external influences that bring about varying expression of the floral mechanisms.

Of the original 14 families, three were selected for further propagation and study. These are numbers 4, 6, and 10. Family 4 showed the least tendency to produce anthers in the terminal inflorescences. Families 6 and 10 produced female plants with a strong tendency to develop male parts both in the terminal inflorescences of the female plants and in the lateral inflorescences of the male plants. Family 6 was cross-pollinated with family 5 and family 10 with 6. All three families were closely

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alike in appearance and in the degree of sex-intergradation. Following this original crossing, the progenies have resulted from matings within the family. Number 4 has been mated within the family from the beginning. Families 6 and 10 showed somewhat more vigor in the first two or three generations following the crossing of these related lines, but in the later generations the plants have made no larger growth than family 4.

The behavior of these three families during five generations is shown in table 1. An arbitrary scale was devised with which to classify the female plants ranging from 0 to 9, depending upon the number of florets in the terminal inflorescence having anthers. In the zero grade all of the flowers are pistillate with no anthers. In grade 1 the florets at the tip of the main spike have a few aborted anthers but do not produce functional pollen. Grade 2 produces a small quantity of functional pollen, and the higher grades have an increasing proportion of anthers and a gradually decreasing number of stigmas up to a complete change of the female to the male condition in the terminal inflorescence.

Before 1935 the female progenitors of these dioecious families were selected for a low grade of terminal inflorescence, either 0 or 1. In 1935 it was noted that families 6 and 10 had a tendency to produce a higher proportion of anthers in the female plants than the others. From then on plants of a high grade, from 5 to 9, were selected as progenitors. Thus families 6 and 10 were selected during three generations for a high degree of sex-intergrades, while family 4 was continued to be selected for a low degree. The result is shown in table 1. There is a slight shift upward in the number and degree of female plants with male tendencies in families 6 and 10. Family 4 on the other hand remained approximately the same throughout the fiveyear period.

The important point is the pronounced difference in degree and variability of intersexuality in the three families. Since they came from the same original plants and have been grown continuously under the same environmental conditions, the con-

Table 1. The behavior of three families of dioecious maize following selection for different grades of floral expression in the terminal inflorescence of the female plants.

177 !1	37			Gra	de of	termi	nal in	flores	cence					Tota	.1
Family number	Year - grown	0	1	2	3	4	5	6	7	8	9	- Average grade	φ	8	₽ &
4	1934	1	20	4	2	1						$1.4 \pm 0.8$	28	30	3
	1935		5	17	17		3					$2.5 \pm 1.0$	42	30	2
	1936		6	23	6							$2.0 \pm 0.6$	41	31	2
	1937		2	6	46	4						$2.9 \pm 0.5$	71	77	3
	1938	1	1	26	104	13	-1					$2.9 \pm 0.6$	168	138	0
	Total	2	34	76	175	18	4					$2.6 \pm 0.8$	350	306	10
6	1934	5	1	1			2			1	1	$2.7 \pm 3.3$	12	11	10
	1935	1		1	1		2				. 1	$4.0 \pm 2.8$	6	4	6
	1936	1	2	4		2			1		2	$3.6 \pm 3.0$	13	16	7
	1937						2	1	1	4	10	$8.1 \pm 1.4$	25	27	0
	1938	1	1		1	2	5	1	2	3	6	$6.1 \pm 2.6$	25	29	4
	Total	8	4	6	2	4	11	2	4	8	20	$5.5 \pm 3.2$	81	87	. 27
10	1934		15			1	2					$1.6 \pm 1.4$	18	8	4
	1935	1			1	1	2	·			1	$4.3 \pm 2.7$	6	0	6
	1936			2	2	3	2					$3.6 \pm 1.1$	10	8	0
	1937			1	2.	3	5	1	2			$4.6 \pm 1.4$	16	17	1
	1938				4.	7	4	3	1		1	$4.7 \pm 1.5$	24	17	0
	Total	1	15	3	9	15	15	4	3		2	$3.7 \pm 2.0$	74	50	11

clusion is obvious that the determining factors are within the plants themselves and associated with the transmissible heredity.

The male plants also have shown a tendency to vary. This is expressed in the usually sterile lateral inflorescence. As illustrated and described in the previous report (Jones, 1934), these lateral inflorescences occasionally produce a few abortive stigmas. Rarely do these extend beyond the enclosing husks (bracts) and are therefore seldom fertilized. In a few cases seeds are produced. When the husks are spread apart and pollen is applied to these poorly developed stigmas, seeds are sometimes formed.

Other plants produce anthers at the tips of these sterile female inflorescences. The anthers never contain well-formed pollen and even when exposed to the air do not dehisce. Stigmas and anthers have never been found in the same lateral inflorescence in these families. In table 2 the three families are compared in the number of male plants that have male or female tendencies in the lateral inflorescences. Family 4 produces a large proportion of female intergrades and no male intergrades. Families 6 and 10, in contrast, produce few individuals with female tendencies and a slightly larger number with male tendencies. Thus the family with the greatest tendency towards femaleness in the female plants also has the most tendency towards femaleness in the male plants. There is therefore a decided shift towards femaleness in this family. In the other two families the shift is in the opposite direction. Both males and females show more male characters.

This is proof that there are modifying factors which govern the expression of the two main sex-differentiating loci. Whether these are carried in the chromosomes or in some other place cannot be

determined from the results obtained so far. Since they segregated at random in the early generations and responded to selection in the later generations, they are presumably in the chromosomes.

The five growing seasons including 1934 and 1938 were characterized by considerable variation in rainfall and temperature. The first three years were drier than normal during some part of the summer, and plants suffered considerably. In 1937 the season was generally favorable. The plants made a luxuriant growth and yielded well. In 1938 the first part of the season was excessively wet and cool. There was much leaching, and plants suffered

Table 2. A comparison of three families of dioecious maize in respect to the floral expression of the lateral inflorescence of the male plants.

			male pla ll inflores	
Family number	Year - grown	φ	8	₽ &
4	1934	18	0	12
	1936	14	0	17
	1937	26	0	51
	1938	21	0	117
	Total	79	0	197
6	1934	0	0	11
	1936	5	3	8
	1937	0	3	24
	1938	0	2	27
	Total	5	8	70
10	1934	0	0	8
	1936	1	0	7
	1937	2	1	14
	1938	1	5	11
	Total	4	6	40

Table 3. A comparison of inbred and intercrossed progenies in family 4 of dioecious maize in respect to the floral expression of the terminal inflorescence of the female plants.

		Gr	ade (	of te	rmin	al in	flore	escen	ce		A a a a	To	tal
Progeny number	0	1	2	3	4	5	6	7	8	9	Average grade	φ	ð
101			1	9								12	10
104		٠.		9	2							13	7
107			2	6			• • •		• •			10	8
110				5				٠				6	14
113				10	1			٠	·			12	. 8
115			2	8				٠				11	
118				7	• •.	1						8	10
121	1		. 1	6								11	
Total of inbred progenies	1	• • •	6	60	3	1					$2.9 \pm 0.6$	83	73
102			٠	5	4							9	1
105				7	4			١				12	
108			4	4								11	. 4
111			1	11								12	
114			10		1							12	
116	٠		1	8								13	
119	· · ·		2	6					٠	• •		8	10
122		1	2	3	1		٠					8	
Total of intercrossed progenie	s	1	20	44	10						$2.8 \pm 0.7$	85	6.

from nitrogen starvation during the later part of the season. The plants in family 4 responded approximately the same to all five seasons. There is a slight shift upward in grade of the terminal inflorescence but not of the same order as in families 6 and 10 where there was a response to selection. The same situation is apparent in table 2 where seasonal fluctuations had no noticeable effect. This is evidence that sex expression in these families of dioecious maize is influenced relatively little by the fluctuations of their normal environment as it may vary in rainfall, temperature, and fertility.

It is also clear from table 3 that sex expression in these plants is not influenced by hybrid vigor. In family 4 eight progenies were grown in 1938 from parental plants that had been mated within the same line by sib-pollinations. Eight other progenies from the same lines were grown from seed that had been intercrossed between different lines, all within the same family. The intercrossed progenies were taller and more vigorous, but there is no difference in the grade of the female plants. These two lots were grown in alternating rows. Since hybrid vigor has much the same effect upon plants as better environmental conditions, this is additional evidence that, in these plants, sex expression is independent of the usual environmental deviations.

Sharp (1934) and Loehwing (1938) have reviewed investigations dealing with external effects upon sex expression in plants. There is much evidence to show that nutritional and other environmental agencies have a modifying influence but only when these changes are extreme. In the normally fluctuating environment these dioecious maize plants are relatively free from external modification and show clearly the action of internal determiners capable of meiotic recombination.

The total number of female, male, and normal monoecious plants is given in table 1. The totals include some plants that could not be classified as to grade due to smut infection or other causes of incomplete development of the terminal inflorescences. As explained in the previous publication, it is not possible to distinguish between female plants which are homozygous sk sk and those which are heterozygous Sk sk. The latter will continue to give some normal bisexual plants. Any outcrossing with unrelated plants will also give normal individuals. Usually these can be detected on account of their larger size. In families 6 and 10 the upper grades of the female plants approach the normal condition and undoubtedly some of the plants classified as hermaphrodites are females that are completely changed to males in the terminal inflorescence. Such plants should still be homozygous silkless, but so far no plants have been identified by testing.

In the three families, 4, 6, and 10, several progenies were grown each year from pollinations of individual female by individual male plants. In 1934 two out of four progenies in family 4 produced no normal hermaphrodites. In 1938 all the 16 progenies grown contained no plants of this type with the exception of one tall vigorous plant that was clearly an outcross. In families 6 and 10 a considerably larger number of hermaphrodites appeared, but some progenies produced none. In 1937 seven female plants in as many different progenies of family 4 were outcrossed by the original silkless strain. The resulting F<sub>1</sub> plants, 140 in all, were completely silkless with normal tassels. Three progenies in family 6 were tested in the same way and produced only silkless plants. Apparently most of the heterozygous Sk sk individuals have been eliminated. If this is true, the progenies that are free from hermaphrodites should remain so providing accidental outcrossing can be prevented.

There has been no significant change in the sexratio during the nine generations this dioecious corn has been continued. As reported in the previous publication, the ratio based on all progenies free from hermaphrodites was 1 male to 1.24 females. From 1934 to 1938 the ratio in family 4 was 1 to 1.14, based on 656 individuals. This same family in 1938 with no hermaphrodites produced 138 males and 168 females, a ratio of 1 to 1.22. The constant excess of females over males cannot be accounted for. There are no visible differences in the chromosomes and no apparent reason why a gamete carrying a chromosome with recessive  $ts_2$  should have any competitive advantage over its homolog carrying the dominant male-determining allele.

#### SUMMARY

Three families of dioecious maize, originating from a combination of known mutant types, have been propagated for nine generations. All families produce some individuals with a varying degree of male characteristics in the female plants and female characteristics in the male plants.

The families differ in the number and character of these sex-intergrades and respond to selection.

Sex expression in this material is relatively uninfluenced by the normally fluctuating environment and appears to be governed by internal factors capable of segregation and recombination.

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## A NEW GROWTH SUBSTANCE, β-NAPHTHOXYACETIC ACID 1

#### S. C. Bausor

MANY SYNTHETIC, hormone-like substances possessing properties that regulate growth and development of plants have been described recently by workers of the Boyce Thompson Institute and others (Zimmerman and Hitchcock, 1937; Zimmerman and Wilcoxon, 1935; Wilcoxon, 1937; Nicol, 1938; Boycen Jensen, 1936; Went and Thimann, 1937; et al.). Among these was α-naphthalene acetic acid which was found to have approximately the same physiological potentialities in causing growth curvatures as 3-indole acetic acid, although Avery et al. (Zimmerman and Hitchcock, 1937) and Thimann and Bonner (1938) showed it to be much less efficient when tested by the Avena coleoptile method. Moreover, its efficiency in inducing root formation on intact plants and cuttings was shown to be greater than that of 3-indole acetic acid (Zimmerman and Hitchcock, 1937). Likewise, a-naphthalene-aceto-nitrile proved physiologically effective, although slower than a-naphthalene acetic acid in its action, but  $\beta$ -naphthalene acetic acid was only slightly active. Acenaphthene acetic acid also showed growth promoting properties (Zimmerman and Wilcoxon, 1935; Nicol, 1938).

MATERIALS AND METHODS.—For the present study the growth-regulating properties of  $\beta$ -naphthoxyacetic acid were tested by applying various concentrations of the material in anhydrous lanolin paste to stems and leaves of intact experimental plants. The substance readily goes into solution in lanolin with gentle heating. The following species were used:  $Ocimum\ Basilicum\ L.,\ Mimosa\ pudica$ ,

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L., Eranthemum nervosum, R. Br., and Lycopersicum esculentum, Mill. Two groups of control plants were used, one treated with lanolin to which nothing was added and the other with a lanolin paste containing 3-indole acetic acid.

Cuttings of Coleus Blumei, Benth., and Taxus cuspidata, Sieb. & Zucc., were treated with aqueous solutions of  $\beta$ -naphthoxyacetic acid and then planted in a sand bed.

Synthesis of  $\beta$ -naphthoxyacetic acid.—The naphthyl ethers are readily formed from naphthol and an alkyl halide (Taylor, 1930; Gilman, 1938):

In the preparation of  $\beta$ -naphthoxyacetic acid,  $\beta$ -naphthol and monochloracetic acid were refluxed in an aqueous solution of sodium or potassium hydroxide. The following is a typical experiment.

Fifty grams of  $\beta$ -naphthol were dissolved in 450 cc. of water containing 24 grams of KOH. In another flask, 32.7 grams of chloracetic acid were dissolved in 250 cc. of water containing 24 grams of KOH. A slight excess of hydroxide was thus used in each case. The two solutions were mixed together,

shaken, and refluxed for one hour. On cooling, a copious precipitate of potassium  $\beta$ -naphthoxyacetate came out of solution. This is only slightly soluble in water or alcohol and can be purified easily. It was converted to the acid by dissolving in acid alcohol and precipitating by dilution with water. In a typical experiment 4 grams of sodium  $\beta$ -naphthoxyacetate were added to 50 cc. of alcohol and shaken. To the slush thus formed, 10 cc. of 5 per cent sulphuric acid in water were added and the precipitate dissolved. Then 30 cc. more of 5 per cent  $H_2SO_4$  were added, making 40 cc. in all. By adding 250 cc. of water,  $\beta$ -naphthoxyacetic acid was precipitated. The yield, calculated on the basis of  $\beta$ -naphthol, is about 55 per cent of the expected.

The melting point of  $\beta$ -naphthoxyacetic acid is

154-156°C.

It might be noted here that both the sodium and potassium salts were found active as growth-regu-

lating substances also.

EXPERIMENTAL RESULTS.—In recording the results, curvatures produced by unilateral application of the lanolin pastes are termed positive when bending is toward the place of application, and negative when away from it, the assumption being that the latter indicates a stimulating effect and the former an inhibiting one (Went and Thimann, 1937).

Stem reactions of Ocimum Basilicum.—Applied to one side of the stem of Ocimum Basilicum a 1 per cent solution of naphthoxyacetic acid in anhydrous lanolin produced strong positive curvatures at the end of 24 hours, but this concentration proved toxic to the tissues it contacted, causing a brown flaccid necrosis at this place. The leaves wilted and turned brownish-black. This toxic effect was very conspicuous about three days after the application. Below the point where the paste was placed, an abundance of roots appeared, in many cases, down to the basal internode. The root primordia were visible as white bulges in about a week.

With a 0.1 per cent paste, applied in the same way, the stems also curved positively, but showed no evidence of toxic effects. In seven days an abundance of roots had developed, not only below the point of application, but also at the place of contact. The rooting response was more active with the 0.1 per cent paste than with the 1 per cent. It appeared that the rooting response with the higher concentrations, which always occurred below the point of contact, came about by dilution of the naphthoxyacetic acid as it diffused downward.

A 1 per cent lanolin paste of 3-indole acetic acid produced none of the toxic symptoms of  $\beta$ -naphthoxyacetic acid. It caused negative curvatures when applied to the stems, and the root-inducing effects were confined to the area contacted by the paste.

The stems curved negatively when a 0.015 per cent paste was used, but at this concentration no roots developed later. At a concentration of 0.001 per cent no effects were observed.

Leaf reactions of Ocimum Basilicum.—When 1 per cent  $\beta$ -naphthoxyacetic acid in lanolin was applied to the upper or lower sides of the petioles, slight positive curvatures resulted in 24 hours which became more pronounced after three days. When a plant was decapitated at a node and the paste applied in the axil of one of the opposite leaves, the treated leaf curved upward positively (hyponasty) and in three days was almost vertical in position. The opposite leaf showed no effect.

The reaction of the petioles was less definite when a 0.1 per cent paste was used but was essentially like that obtained with the 1 per cent paste.

When a 0.1 per cent paste was applied to the blade of the leaves, no curvature of the petioles resulted in three days. In an experiment in which half of the lower surface of the blade was coated, a positive curvature of the petiole was noted eight days after application. In addition to the down-bending of the petiole, the internode below showed a positive curvature also, and on the side of the petiole corresponding to the side of the lamina on which the lanolin was smeared, three root primordia developed.

Slight negative curvatures resulted in 24 hours when a 0.015 per cent concentration of the paste was applied to either the upper or lower side of the petiole. A 0.001 per cent paste showed no effect.

The basil plants upon which these experiments were performed were about eight inches tall and

grown in pots.

Stem reactions of Mimosa pudica.—A 1 per cent lanolin paste of naphthoxyacetic acid applied to one side of the apical peduncle, on which the flowers had not expanded, produced a strong positive curvature of the apex when observed three and one-half hours after treatment. In 24 hours the curvature was still greater. Two additional peduncles, one at the first node and the other at the second node from the apex, curved downward, although not contacted by the paste. A 0.1 per cent paste similarly applied to an apical peduncle also resulted in a strong positive curvature of the apex. When 0.015 per cent was used, a very slight negative curvature resulted which was not noticeable after three days. No reactions followed treatment with 0.001 per cent.

When a 1 per cent paste was applied to a peduncle with expanded flowers, a strong positive curvature was produced, while pastes containing 0.1, 0.015, and 0.001 per cent, when similarly applied to peduncles bearing open flowers, produced small negative curvatures. It is interesting to note that the peduncle of *Mimosa* was the only organ tested which reacted to a concentration as low as 0.001 per cent.

In no case did rooting occur in Mimosa as a result of these treatments.

Leaf reactions of Mimosa pudica.—A 1 per cent lanolin paste of naphthoxyacetic acid applied to the lower side of the petiole of an unexpanded leaf—but not at the pulvinus—resulted in a strong positive curvature. When the experiment was termi-

nated after nine days, this leaf was dying, indicating that the material was toxic. On the other hand, a 0.1 per cent concentration produced very strong negative curvatures in the petioles of unexpanded leaves, and similar negative curvatures were observed with a 0.015 per cent paste three and one-half hours after application, but the curvature disappeared after 24 hours when the next observation was made. A 0.001 per cent paste showed no effect on the petioles of unexpanded leaves.

When a 1 per cent lanolin paste was applied to the upper side of the pulvinus of a mature leaf, there was no apparent effect. However, on stimulating the leaf by touching it, the leaflets folded together normally, but the petiole did not respond by dropping. This effect remained the same during nine days of observation. A similar effect was produced with a concentration of 0.1 per cent and persisted for several days. But at the end of the nine days' duration of the experiment, the petiole dropped permanently. Concentrations of 0.015 and 0.001 per cent similarly applied to pulvini of mature leaves had no effect on the normal response of the leaves.

When applied to the upper or lower sides—not at the pulvini—of petioles of mature leaves, no effects were produced by concentrations of 0.1, 0.015, and 0.001 per cent, and the leaves responded normally to stimulation.

No effects other than necrosis resulted when the lanolin pastes were applied directly to either the upper or lower surfaces of the apical leaflets.

Burkholder and Pratt (1936) described the effect of 3-indole acetic acid upon Mimosa leaves. They reported that the petioles moved upward and the leaflets remained closed when a lanolin paste of a small concentration of 3-indole acetic acid was applied to the lower surface of the pulvinus and that the petiole moved downward and the leaflets remained open when the paste was located on the upper side.

Reactions of Eranthemum nervosum.—Both 1 and 0.1 per cent pastes of  $\beta$ -naphthoxyacetic acid in lanolin applied to the under surface of the petiole of the leaf caused that organ to curve downward, reaching a maximum curvature about the third day. The internode below the treated leaf was affected also and curved negatively, assuming that the material diffused down the stem on the same side to which the leaf was attached—i.e., it curved away from the leaf. 0.015 and 0.001 per cent pastes had no effect when applied in the same way.

In another series of experiments, the pastes were smeared on one-half of the upper surface of the lamina of the leaf on one side of the midrib. With a concentration of 1 per cent the petiole curved downward and bent horizontally away from the treated side of the blade. The blade itself showed no obvious effects. A 0.1 per cent paste produced a similar reaction but much less pronounced, while both 0.015 per cent and 0.001 per cent pastes were inert.

Reactions of Lycopersicum esculentum.—Only a few tomato plants were available at the time these experiments were performed, but the few trial runs induced the following responses to  $\beta$ -naphthoxy-acetic acid; when a 1 per cent lanolin paste was applied to the stem, it caused a strong positive curvature and epinasty of the leaves. Root primordia later appeared in great abundance in large swollen areas along the stem. A dilute aqueous solution applied to the soil caused an epinastic response of the leaves followed by induction of root primordia similar to the response described by Hitchcock and Zimmerman (1935) for a-naphthalene acetic acid, etc.

A lanolin paste of sodium naphthoxyacetate (saturated) applied to the stem caused a negative curvature and epinasty. It is interesting to note that the action of the salt is slow as compared to that of the acid, and large quantities of it can be applied without toxic effects, presumably because of its low solubility. Further work on the properties of the salts is in progress.

Cuttings.—The effectiveness of aqueous solutions of  $\beta$ -naphthoxyacetic acid in causing root formation was tested according to the method used by Hitchcock and Zimmerman (1936). Cuttings of Coleus Blumei were treated for 24 hours in an aqueous solution of naphthoxyacetic acid,—10 mg. per 100 cc.,—while controls were placed in tap water for the same length of time. In a week both lots had rooted, and a comparison showed more abundant but thinner roots on the experimental plants.

Cuttings of Taxus cuspidata treated with 10 mg., 5 mg., and 2 mg. of naphthoxyacetic acid per 100 cc. of tap water for 24 hours and controlled by cuttings similarly treated with 3-indole acetic acid, and also untreated cuttings, gave inconclusive results. In six weeks all lots had a well-developed callus and were about equally well rooted.

Discussion.—The data presented here indicate that  $\beta$ -naphthoxyacetic acid evokes physiological responses in plants similar to those of the numerous synthetic growth substances already reported. (Zimmerman and Hitchcock have reported on about fifty such substances.)

 $\beta$ -naphthoxyacetic acid is more toxic than its close relative,  $\alpha$ -naphthalene acetic acid, producing detrimental effects at a concentration as low as 1 per cent, especially in herbaceous plants. Another specific difference lies in its inducing positive curvatures, thus inhibiting growth by elongation, at concentrations which appear to be optimum for root induction.

Koefli, Thimann, and Went (1937) re-examined the properties of most of the known growth substances and brought forward the generalization that a closed ring system with double bonds and a side chain containing a carboxyl group at least one carbon atom removed from the ring were necessary constituents of a growth substance. Both a-naphthalene acetic acid and  $\beta$ -naphthoxyacetic acid fall

within these minimum requirements for a growth substance

The fundamental characteristics of growth substances are exhibited by both compounds, although they differ in specific reactions, as might be expected. It would seem that the only rational conclusion to be drawn is that proposed by Söding (1936) and accepted by Zimmerman, Hitchcock, and Wilcoxon (1936) that growth substances are nonspecific.

The reaction of naphthoxyacetic acid upon the petiole of *Mimosa* is noteworthy, since it seems to affect the permeability of the parenchyma of the pulvinus. This is inferred from the fact that the response of the leaf is modified, although there are no other apparent changes. However, a direct investigation on this point was not made, and this must therefore stand as a conjecture.

#### SUMMARY

The properties of  $\beta$ -naphthoxyacetic acid are described and its synthesis detailed. Its activity was tested by means of various concentrations in lanolin

paste applied to intact stems and leaves of Ocimum Basilicum, L., Mimosa pudica, L., Eranthemum nervosum, R. Br., and Lycopersicum esculentum. Mill. Evidence of the diffusibility and physiological activity of β-naphthoxyacetic acid was shown in all of them. Curvatures of stems and leaves resulted from unilateral treatment and in Ocimum and Lycopersicum were subsequently followed by the formation of roots over large areas of the stem. A 0.1 per cent paste proved optimum for this rooting response. In Mimosa pudica an inhibiting effect of the substance on the normal reaction of the pulvini was observed, which prevented the petiole from dropping after stimulation. Cuttings of Coleus Blumei, Benth., and Taxus cuspidata, Sieb. & Zucc., treated with an aqueous solution of the material resulted in more abundant roots in the former as the result of treatment, while the results with the latter were inconclusive.

Sodium and potassium  $\beta$ -naphthoxyacetate also were found to be active.

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## STUDIES IN THE GENUS LESQUERELLA 1

### Reed C. Rollins

PAYSON IN 1922 completed a thoroughly creditable monograph of Lesquerella which is the standard reference on the genus. Since then Fernald (1933), Munz (1929, 1932), Wiggins (1933), Peck (1934), and Cory (1930) have added new species, which together with those proposed in the present paper strongly indicate that this chiefly west-American genus is not entirely known even now. During several field seasons material of Lesquerella was collected for taxonomic and cytological investigation. The results of a study of this material, together with the description of a new variety collected in Alaska by Miss Edith Scamman, form the basis for the present report. The chromosome numbers summarized in table 1 were determined from aceto-carmen smears of root-tips and developing pollen. Specimens of each collection are in the Gray Herbarium of Harvard University.

These data are not sufficient to form the basis for any general conclusions regarding the cytology of Lesquerella, but two significant points become clear. First, there is an apparent aneuploid relationship between species of Lesquerella, a condition not commonly found in the Cruciferae. According to Manton (1932), aneuploidy between species in the familv is found chiefly in the Brassicinae. Second, polyploidy occurs naturally in at least some species of the genus. It is interesting to note that the polyploid L. ludoviciana from Mesa County, Colorado, exhibits the typical "gigas" characteristics often associated with plants possessing a multiple genom. Plants of this collection are several decimeters taller; the pedicels are more remote in the inforescence; the stellae are larger and form a less dense cover on plant parts, and the siliques are slightly larger than in the usual form of the species.

Several species of Lesquerella have been grown in the greenhouse for the purpose of observing their ontogenetic development and the effect of altered environmental conditions on the disposition of the indument over the plant surface. Those familiar

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with section Eulesquerella of the genus are aware that the plants of most species are literally encrusted with a dense covering of nearly orbicular stellae. It is a current problem in dealing with the systematic position of many varieties and species in the Cruciferae as to how much weight can be given to the presence or absence of an indumentum. Thus, by demonstrating the stability or lack of stability of this feature, some clue as to its usefulness as a taxonomic criterion should be obtained. In L. arctica var. Scammanae, L. subumbellata, L. intermedia, L. alpina, L. ovalifolia, and L. calcicola greenhouse plants were critically examined and compared with the specimens from which seed had been obtained in each instance. Particular attention was given to the abundance and disposition of the stellae. These were found to differ in no discernible way in the same species whether they were greenhouse grown or came from their natural habitat. From this we may roughly conclude that under greenhouse conditions of reduced sunlight and increased humidity, the abundance and disposition of the indument of Lesquerella is unaltered in firstgeneration plants. In connection with these observations, it was interesting to note that the cotyledons were in every case perfectly glabrous. The first pair of true leaves were always densely encrusted with stellae as in the later foliage.

The following new entities have been discovered in Lesquerella in the past several years.

Lesquerella calcicola sp. nov. (fig. 1A, B).—Perennial, caespitose, silvery stellate-pubescent throughout; caudex simple or closely branching, elongated, often producing sterile shoots; stellae small, closely appressed, rays numerous and coherent at base; stems coarse, erect, simple, 1–3 dm. long; basal leaves numerous, petiolate, linear-oblanceolate, acute, flat, 4–10 cm. long, 4–6 mm. wide; cauline similar, 2–4 cm. long, 3–5 mm. wide; inflorescence racemose, congested; pedicels stout, spreading with a strongly sigmoid curve upward, 1–1.5 cm. long; petals yellow, spatulate, retuse, 9–11 mm. long; sepals linear-oblong, inrolled at apex, 4–6 mm. long, 2 mm. wide; siliques sessile, oblong or oval in outline, erect, com-

TABLE 1.

Name of plant	Place of collection	Collector and number		Chromosome number	
				N	2N
Lesquerella alpina	Uinta Co., Wyoming	Rollins	1684	5	10
L. montana	Chaffee Co., Colorado	Rollins	2078	5	
L. montana var. suffrutescens	Las Animas Co., Colorado	Rollins	1816	5	
L. calcicola	Las Animas Co., Colorado	Rollins	1861		ca. 20
L. Fendleri	Las Animas Co., Colorado	Rollins	1825		12
L. Fendleri	Bent Co., Colorado	Rollins	2054	6	
L. Fendleri	Pueblo Co., Colorado	Rollins	2065	6	
L. intermedia	Fremont Co., Colorado	Rollins	2067	8	
L. ludoviciana	Mesa Co., Colorado	Rollins	2171	15	
L. ludoviciana	Uinta Co., Wyoming	Rollins	2237	5	

pressed at apex, stellate-pubescent, 6-9 mm. long, 4-5 mm. wide; style 4-5 mm. long, stigma expanded; ovules 2-4 in each loculus; seeds orbicular, wingless, ca. 3 mm. broad; cotyledons accumbent.

Herba perennis caespitosa undique indumento argenteo-stellato tecta; caulibus erectis vel procumbentibus simplicibus; foliis radicalibus lineari-oblanceolatis acutis 4–10 cm. longis, 4–6 mm. latis; pedicellis divaricatis 1–1.5 cm. longis; floribus subluteis racemosis congestis; siliculis oblongis vel ovatis stellato-pubescentibus 6–9 mm. longis; stylo 4–5 mm. longo; loculis 2–4-ovulatis; seminibus immarginatis ca. 3 mm. latis.

Colorado: limestone or gypsum outcrop, 3 miles east of San Francisco Creek, 8 miles northwest of Trinchera, Las Animas County, July 13, 1937, Rollins 1861 (type in Gray Herb.); 10 miles south of Pueblo, Pueblo County, July, 1937, Rollins 1799 (G). New Mexico: Emery Gap to Branson, Colorado, Union County, June, 1924, Eggleston 20148 (G).

Lesquerella calcicola is nearest related to L. intermedia from which it differs in having broader, petiolate, non-involute basal leaves which are 4-10 cm. long. The basal leaves of L. intermedia are nar-

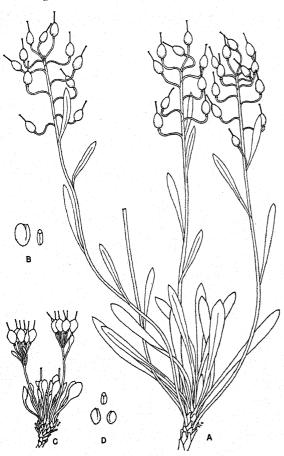


Fig. 1.—A, B. Lesquerella calcicola Rollins, drawn from Rollins 1861; A, habit sketch,  $\times \frac{1}{2}$ ; B, seed in broad view and transverse section,  $\times 2$ .—C, D. Lesquerella subumbellata Rollins, drawn from Rollins 1758; C, habit sketch,  $\times 1$ ; D, seed in broad view and transverse section,  $\times 2$ .

rowly linear, involute and only 1-4 cm. long. The pedicels of L. calcicola are definitely sigmoid, whereas those of L. intermedia are straight or nearly so. L. calcicola has pods which are oval to oblong, compressed at apex and 6-9 mm. long; in L. intermedia the silique is nearly round or slightly oval, uncompressed at apex and 4-6 mm. long. There are 2-4 ovules in each loculus of the former species and 3-8 in each loculus of the latter. L. calcicola is a taller, more robust plant with sterile soboles terminating the main axis; in L. intermedia the growing point usually produces a fertile shoot.

The types of those species of Lesquerella related to L. calcicola have been studied, with one exception. Specimens of L. valida Greene have not been available for examination directly. The original description of this species leaves little doubt that it differs widely from L. calcicola. However, in order to be doubly sure that no error was being made in describing my plants as new, specimens were transmitted to Dr. Theodor Just, who kindly compared them with the type of L. valida in Greene's herbarium at the University of Notre Dame. Dr. Just concluded that the most conspicuous differences between these species were to be found in the shape and dimensions of the foliage, but that these were by no means the only points of divergence. The basal leaves of L. valida are obovate to broadly spatulate with an obtuse apex and only 1-2.5 cm. long. Actually the two species appear not to be very close relatives.

LESQUERELLA subumbellata sp. nov. (fig. 1C, D).-Perennial, silvery stellate-pubescent throughout; stellae small, closely appressed, rays numerous, forked, irregularly coherent at base; caudex closely branched; stems erect, few to several, slender, simple, 1-3 cm. long; basal leaves numerous, linear to linear-oblanceolate, 1-2 cm. long; blade expanded, obtuse or acute, tapering abruptly to a long petiole; cauline one or two, similar to basal; pedicels spreading, nearly straight, 3-6 mm. long; inflorescence corymbose, appearing umbellate in fruit; sepals oblong, narrowed to an acute apex, 2.5-4 mm. long, 1-1.5 mm. wide; petals yellow, lingulate, retuse, 4-7 mm. long, 1.5-2.5 mm. wide; filaments slender, abruptly dilated at base; siliques sessile, obovate in outline, flattened parallel to partition, densely pubescent, 2.5-3.5 mm. long, nearly as broad, vacant apex flattened; style purplish, 1.5-2.5 mm. long, stigma unexpanded; seeds marginless, brown, orbicular to slightly oblong, ca. 2 mm. long; ovules two in each loculus, funiculi attached for one-fourth their length; cotyledons accumbent.

Herba perennis undique indumento argenteo-stellato tecta; caulibus tenuissimis erectis simplicibus 1-3 cm. longis; foliis radicalibus integris lineari-oblanceolatis 1-2 cm. longis; floribus paucis subluteis corymbose dispositis; siliculis obovatis vel subglobosis sessilibus stellato-pubescentibus 2.5-3.5 mm. longis; stylis subrubris 1.5-2.5 mm. longis; loculis 2-ovulatis; seminibus immarginatis ca. 2 mm. longis.

Utah: limy knoll-crest, juniper belt, foothills of the Uinta Mountains, 18 miles north of Vernal, Uintah County, June 17, 1937, Rollins 1758 (type in Gray Herb.); south of La Point Road, 4 miles southwest of Vernal, May, 1933, Graham 7833 (G).

This species is most closely related to L. condensata and its variety laevis but differs from both in a number of significant ways. The stellae of L. subumbellata are small, short-rayed, and closely appressed; the pedicels are straight; the siliques are obovate with obtuse apex, 2.5-3.5 mm. long, and the purplish style which is about 2 mm. long has an unexpanded stigma. In L. condensata the stellae are larger, with long, distinct rays; the pedicels tend to become sigmoid; the ovate to globose siliques have a tapering apex and are 3-5 mm. long. The style which is 2-3 mm. long has an expanded stigma. These species differ in habit of growth, L. subumbellata having slender, well-exserted stems and a much contracted delicate caudex instead of shortened stems which are virtually buried in a pulvinate cushion of leaves as in L. condensata. The latter species has a robust, muchbranched caudex which is usually subterranean. Geographically, L. condensata and var. laevis are found on the west and east side of the Continental Divide in Wyoming and Montana. L. subumbellata is apparently endemic in the Uintah Basin of Utah.

LESQUERELLA ARCTICA (Wormsk.) Watson, var. Scammanae var. nov.—Fruiting stems 1-3 dm. long, mostly erect; basal leaves spatulate, tapering to a long narrowed petiole, 1-2 cm. broad, 5-15 cm. long; fruiting pedicels 1.5-4 cm. long; silique slightly elongate, nearly glabrous, scattered stellae often present; style 2-3 mm. long.

Herba perennis; caulibus 1-3 dm. longis; foliis radicalibus 5-15 cm. longis, 1-2 cm. latis; pedicellis erectis 1.5-4 cm. longis; stylis 2-3 mm. longis.

Alaska: Rapids Lodge, 138 miles south of Fairbanks on Richardson Highway, August 7-10, 1936, Edith Scamman 216 (type in Gray Herb.); August 25-28, 1937, Edith Scamman 1000 (G).

Even when the widest natural variation in Lesquerella arctica is taken into account, the variety here proposed appears amply distinct as a subunit. Variety Scammanae has stems, leaves, and pedicels nearly twice the length of those in the species proper. The new plant is isolated from the typical species in the interior of Alaska.

The question of the proper use of the specific epithet argentea in the genus Lesquerella has recently come to my attention. In this connection, it should be pointed out that the earliest use of Lesquerella argentea was that of Watson (1888) based on Vesicaria argentea Schauer. Payson (1922) failed to recognize this combination as valid presumably because Watson placed a parenthetical question mark between the generic and specific name when the original combination was made. This, it seems to me, must be interpreted as biological doubt rather than nomenclatorial hesitancy; therefore, the name cannot be rejected on that account. With L. argentea (Schauer) Watson, accepted as a valid name, then L. argentea (Pursh) MacMillan (1892) based on Myagrum argenteum Pursh (1814), must be rejected in favor of L. ludoviciana (Nuttall) Watson (op. cit.), which is based on Alyssum ludovicianum Nuttall (1818). Payson (op. cit.) assumed L. ludoviciana to be rare west of the Continental Divide, but I have seen plants of this species growing in abundance in western Wyoming, western Colorado, and eastern Utah. Graham (1937) has reported it to be a frequent plant of low and mid-altitude dry places in the Uintah Basin of Utah.

#### SUMMARY

Chromosome numbers are presented for six species and one variety of Lesquerella, based on the examination of eleven samples of material collected from naturally occurring plants. From these data it is concluded that aneuploidy exists between certain species and that natural polyploidy is present in at least one species of the genus. The indument on plants of five species and one variety of Lesquerella grown in the greenhouse was found to be similarly disposed and equally abundant as on plants of the same entities obtained from their natural habitats. Two new species and one new variety are proposed in the genus Lesquerella.

SOCIETY OF FELLOWS OF HARVARD UNIVERSITY, CAMBRIDGE, MASSACHUSETTS

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## PRODUCTION OF GROWTH SUBSTANCE BY BACTERIA IN MEDIA CONTAINING SPECIFIC ORGANIC AND INORGANIC NITROGENOUS COMPOUNDS <sup>1</sup>

Paul R. Burkholder

The formation of growth-promoting substance (auxin) by microorganisms has been observed in recent years by a number of investigators, but comparatively little knowledge exists concerning the relationship between definite compounds present in the substrate and elaboration of these special substances. In this study pure cultures of Aerobacter aerogenes and Escherichia coli were grown in synthetic media containing single amino acids<sup>2</sup> or inorganic salts as the only source of nitrogen. After periods of incubation, the cultures were tested for growth substance according to a standard Avena coleoptile curvature method commonly used for detection of phytohormones causing cell enlargement.

METHODS.—The culture medium employed for growing the bacteria was glycerol agar made up according to the formula used by Koser and Rettger (1919). Its constitution is as follows: NaCl, 5.0 g.; MgSO<sub>4</sub>, 0.2 g.; CaCl<sub>2</sub>, 0.1 g.; KH<sub>2</sub>PO<sub>4</sub>, 1.0 g.; K<sub>2</sub>HPO<sub>4</sub>, 1.0 g.; glycerol, 30.0 g.; distilled water, 1,000 g.

Sufficient agar was added to make a 2 per cent mixture. Nitrogen was supplied to the cultures by adding a single amino acid to the substrate at the rate of 1 gram per 1,000 cc. of medium. In experiments with inorganic sources of nitrogen the composition of the medium was similar, but 1 gram of NH<sub>4</sub>Cl or of KNO<sub>3</sub> was used per liter, and 4.0 grams of NaCl were added instead of 5.0 grams per liter. All media were put into pyrex culture tubes and sterilized in an autoclave. Only 2 cc. of medium were used in each tube, and the agar was allowed to solidify in an extremely slanted position, so as to permit considerable aeration of the culture during the period of growth. The tubes were inoculated from ordinary nutrient agar slant cultures of A. aerogenes and E. coli, using a small amount of inoculum.

The inoculated tubes were kept in a moist incubator at 33.0°C. for about two or four weeks. Sterile tubes of media were held as controls along with the cultures. During the period of incubation, some of the cultures developed pronounced colors and heavy growths of bacteria, while others remained less colored or showed less growth. At the time selected for making tests for growth substance, agar slants containing the bacteria as well as sterile control tubes of the same media were melted in a water bath, and from each sample agar plates were cast in a brass mold. Great care was exercised in keeping all preparations clean and free from contamination during the testing procedure. The standard size

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 These amino acids were supplied by the Dow Chemical Company to the Department of Botany, Connecticut College, where part of the experimental work was performed. agar plate was cut into twelve pieces and then tested on one dozen Avena coleoptiles according to methods published elsewhere (Avery, Burkholder, and Creighton, 1937).

In certain instances—e.g., with tryptophane—unexpected curvatures were found, and further dilutions of the preparation (originally 1 part of amino acid in 1,000 of medium) were made at the time of testing with pure aqueous agar to yield theoretically one part of the original amino acid in 10,000, 100,000, and 1,000,000 of prepared agar. Preliminary work indicated that with the other amino acids tests could be made satisfactorily without dilution, using the agar as it was made up in the cultures.

RESULTS WITH TRYPTOPHANE.—The results with tryptophane were somewhat different from those obtained with other compounds. A brilliant red color was formed in the medium supporting a rich growth of *E. coli*, while in the cultures of *A. aerogenes* the color was very dark brown at the end of the growth period. Cultures of both organisms in agar supplied with 1 part of tryptophane in 1,000

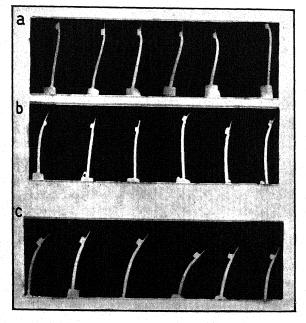


Fig. 1. Curvatures of Avena coleoptiles treated with agar blocks from cultures of A. aerogenes: a, curvatures toward the blocks indicating growth inhibition with an undiluted (1/1,000) tryptophane culture; b, curvatures away from the blocks indicating growth promotion with a diluted (1/100,000) tryptophane agar culture; c, curvatures indicating the presence of growth promoting substance formed in NH<sub>4</sub>Cl agar cultures.

parts of medium produced Avena curvatures toward the applied agar blocks (fig. 1a). This response indicates that growth of the oat tissues in the region immediately below the source of growth substance supply was less than in the opposite side of the coleoptile. Further dilution of the cultures with pure agar vielded test curvatures which were away from the blocks, indicating greater growth in the side under treatment (fig. 1b). The accompanying table 1 gives the values of the curvatures resulting from tests on several different concentrations of substrate made up by diluting the media at the conclusion of the growth period as indicated. It should be noted that in older (30 days) cultures of A. aerogenes the extent of growth inhibition at higher concentrations was somewhat greater than that found in young (14 days) cultures.

Tryptophane agar which had not been autoclaved gave no curvature when tested fresh. Appreciable curves could be obtained with the sterile medium which had been autoclaved and allowed to stand in the incubator for 2 weeks. Sterile autoclaved tryptophane agar originally containing 1/1,000 of the amino acid yielded upon two separate occasions growth promoting curvatures which averaged 4.5° and 4.0°. It may be concluded that heat treatment converts this amino acid to some extent into an active compound. The activity subsequent to autoclaving was never found to be as great as that produced by the action of bacteria growing on the media

Other investigations on tryptophane activation gave some additional information. A pH series of solutions of tryptophane 1/1,000 in water was set up by adding appropriate amounts of HCl or KOH to give the following pH values: 2.0, 3.0, 4.4, 6.9 (tryptophane only in water), and 9.4. After standing at 33°C. for 14 hours, these solutions were mixed individually with equal parts of 3 per cent agar and tested on Avena coleoptiles in a standard manner. No curvatures were found in the test plants after two hours. Apparently the activation of tryptophane in solution is not primarily dependent upon hydrogen-ion concentration, and acidification of the medium in the course of metabolic action of the bacteria would not account for the results obtained.

Further work indicates that temporary activation of tryptophane in aqueous solution takes place, but

the growth promoting property is gradually lost with time. Using a straight growth test method with Avena coleoptiles, as has been employed by Scheer (1937), Miss Genevieve Sargent was able to demonstrate with 1/1,000 tryptophane a spontaneous, but temporary, activation at room temperature.

With the Avena curvature method it was possible to demonstrate complete inactivity of freshly made aqueous solutions of tryptophane. However, after this solution had stood for about four hours at 80°F., curvatures of 3.6 degrees were obtained during the test period of two hours immediately following. Upon further standing in a test tube for 16 or 19 hours, this growth promoting property of the tryptophane solution was lost. Since the activity of tryptophane cultures of bacteria which stood for several weeks was higher than that of sterile control media, it may be concluded that the microorganisms exerted some kind of activating influence which resulted in the formation of a comparatively stable growth substance.

The question arose as to whether or not bacterial cells or the aqueous filtrate from bacteria would be active or capable of activating tryptophane and other amino acids within a short period of time. Tests were made by the Avena curvature technique using agar impregnated with a heavy suspension of A. aerogenes and E. coli, which had been grown on peptone agar, washed with a small amount of distilled water, and finally filtered with a Mandler filter. No curvatures were obtained with agar containing these suspended organisms, nor with the filtered water extract made up in agar. A portion of the aqueous extract was allowed to stand for 7 hours in the presence of an equal volume of 1/1,000 tryptophane. Tests made on this mixture as well as on the amino acid alone in agar gave no indication of growth substance activity. Furthermore, the bacterial extract standing for 7 hours in the presence of proline, cystine, and aspartic acid vielded no curvatures when tests were made on the mixture.

RESULTS WITH OTHER AMINO ACIDS.—Averages of the growth curvatures resulting with 16 different nitrogenous compounds which had supported growth of the two species of bacteria are presented in table 2. The sterile media showed no growth promoting activity when stored in tubes for the same periods of time as the inoculated media. Some of the com-

Table 1. Avena curvatures (in degrees) obtained with A. aerogenes and E. coli cultures on trptophane agar and tested at four different dilutions.

		Tryptophane			
Organism	1/1,000	1/10,000	1/100,000	1/1,000,000	Age of culture
A. aerogenes	{ 9.5° {Retarding	9.0° Retarding	12.3° Promoting	12.0° Promoting	30 days
A. aerogenes	\ 2.5° \ Retarding	3.0° Promoting	9.2° Promoting		14 days
E. coli	\ 2.6°\\ Retarding	4.6° Promoting	15.4° Promoting	•••••	14 days

Table 2. Avena curvatures (in degrees) obtained with cultures of A. aerogenes and E. coli grown in media containing single amino acids.

	Curvatures with cultures		Curvatures with	Age of cultures		
Amino acid	A. aerogenes	E. coli	sterile media	A. aerogenes	E. coli	
	Degrees	Degrees		Days	Days	
d, l-alanine	6.2	8.7	0	24	21	
d-arginine		11.3	0	24	21	
l-asparagine	2.2	7.5	0	24	21	
aspartic acid		13.1	0	15	15	
creatine	•	14.1	0	13	15	
d, l-cystine		16.2	0	16	17	
glutamic acid		8.0	0	13	13	
glycine		10.2	0	15	15	
l-histidine		9.0	0	13	13	
l-leucine		2.8	0	13	13	
leucylglycine		10.2	0	13	13	
methionine		10.5	0	13	13	
l-phenylalanine		5.2	0	13	21	
l-proline		15.1	0	13	21	
l-tyrosine		8.6	0	13	15	
l-valine		2.6	0	14	13	

pounds—e.g., l-valine—were but slightly activated by either of the two organisms. The majority of the substrata showed considerable activation by both organisms, but some yielded greater activity with one organism than with the other. In the case of methionine, E. coli gave high activity, while A. aerogenes was apparently inactive, a result possibly associated with poor growth of the latter in this medium.

For the purpose of finding the effect of concentration of the activated materials upon the curvatures obtained in the tests, series of dilutions of seven amino acid media were made at the time of testing. Results of these observations are given in table 3.

In contrast with the results obtained with tryptophane, dilution of these seven amino acid substrata gave curvatures indicating growth promotion, varying in degree directly with concentration of the media. Maximum responses were found with the highest concentration of 1/1,000 and less with dilu-

tion to 1/10,000; with further dilution to 1/100,000 or beyond, all detectable activity disappeared. These data, presented in table 3, represent the results on separate cultures from those reported in table 2, thus serving to substantiate the findings.

INORGANIC NITROGEN.—During the course of these experiments the question arose as to whether bacteria are able to produce growth promoting substance from materials present in media containing simple compounds of nitrogen. Cultures of A. aerogenes obtained from Dr. N. R. Ziegler, University of Missouri, were grown on basal glycerol agar in which ammonium chloride or potassium nitrate formed the sole source of nitrogen. Inoculated cultures and sterile media were allowed to incubate at 33°C. for one week, and then tests for growth substance were made directly on the agar in the usual way with Avena coleoptiles.

In these tests the sterile substrata gave no curvatures. A. aerogenes cultures on NH<sub>4</sub>Cl yielded curvatures averaging 17.6 degrees (see fig. 1c).

Table 3. Avena curvatures (in degrees) obtained with A. aerogenes cultured in several different amino acid media tested at four different dilutions.

	Dilution	Age of				
Amino acid in medium	1/1,000	1/10,000	1/100,000	1/1,000,000	culture	
	Degrees	Degrees	Degrees	Degrees	Days	
l-asparagine	12.3	6.5	0	0	22	
l-histidine	10.1	3.6	0	0	22	
l-phenylalanine	10.4	5.6	0	0	22	
l-leucine	13.5	5.8	0	0	22	
glycine	6.5	2.0	0	0	28	
d-arginine	13.3	8.3	0	0	28	
l-alanine	10.5	4.3	0	0	28	

Cultures on KNO3 gave 11.3 degrees of curvature. This same organism produced appreciable amounts of growth substance, also, when cultured on the basal medium containing asparagin as the sole source of nitrogen.

It seemed desirable to test for possible transfer of any appreciable amount of growth substance in making inoculations on the culture media containing inorganic nitrogen. Accordingly, A. aerogenes was allowed to form a luxuriant growth upon peptone nutrient agar slants. A large quantity of bacterial growth (many times the amount used as inoculum in making ordinary transfers) was mixed with pure aqueous 2 per cent agar, and tests were made in the usual way with Avena coleoptiles. The test plants behaved just as when treated with plain agar blocks, and it may be concluded, therefore, that in making the inoculations no detectable amount of growth substance was transferred from the cultures on peptone.

Discussion.—Formation of certain growth-promoting substances by microorganisms and their chemical nature has been studied by a number of investigators. Brief reference to some of the outstanding facts will be made here. More than 50 years ago, E. and H. Salkowski (1880, 1885a, 1885b) observed the formation of indole acetic acid and related compounds during the decomposition of protein by mixed cultures of bacteria. Hopkins and Cole (1903) reported on the appearance of indole acetic acid in cultures of Escherichia coli grown in the presence of tryptophane. Herter (1908) pointed out that indole acetic acid, derived from bacterial activity on the intestinal contents, may be excreted in urine, and the following year (Herter and Ten Brock, 1909) published an account of the formation of this substance in cultures of Proteus vulgaris grown on peptone and beef extract. Frieber (1921) described the formation of indole acetic acid from tryptophane in cultures of indole forming bacteria, when sugar is present as a preferred source of carbon; in the absence of sugar, it appears that tryptophane may be converted to indole. Apparently, indole-negative bacteria may form indole acetic acid from tryptophane, whether or not sugar is available. Discussion of other work on the subject may be found in the textbook by Gilman (1938).

New impetus was given to the search for growth regulating substances in plants when the properties of auxins were discovered and quantitative methods for their detection were worked out. Nielsen (1930) found in Rhizopus cultures a new growth regulating substances which he named rhizopin. Then Kögl and Kostermans (1934) succeeded in isolating the highly active indole acetic acid from yeast. Thimann (1935) carried through a number of purification stages the active substance from cultures of Rhizopus suinus; it was shown that formation of indole acetic acid by this fungus grown upon peptone was governed by the amount of tryptophane present and the extent of aeration. The probable production of indole acetic acid by oxidative deamination of tryptophane has been pointed out clearly by Went and Thimann (1937). Boysen Jensen (1932) showed that Aspergillus niger produces active substances from histidine, lysine, leucine, tyrosine, phenylalanine, and tryptophone, but not from glycine, alanine, asparagine, cystine, proline, or arginine. This same author (Boysen Jensen, 1931a) observed the production of a substance active on Avena in cultures of a number of species of bacteria grown on peptone media. Brown and Gardner (1936) obtained evidence for the formation of growth substance by B. tumefaciens.

Thimann (1936) found that root nodules containing R. leguminosarum are centers of growth substance formation. Production of growth substance by clover nodule bacteria grown in yeast water medium plus 0.02 per cent tryptophane has been reported by Chen (1938); no growth substance was found in a culture of nitrogen fixing bacteria grown in the medium without tryptophane. Still more recently Georgi and Beguin (1939) have reported on the elaboration of growth substance by four species of Rhizobium and B. radiobacter cultured on a synthetic medium containing mannitol, mineral salts, and 0.05 per cent tryptophane. All these bacteria were able to produce special substances, in media containing tryptophane, which gave curvature in the Avena test. With mannitol as the source of carbon and KNO3 as the sole source of nitrogen (tryptophane not added), no active substances were produced in the medium.

Few of the earlier workers held any notion whatsoever concerning the possible rôle of bacterial products in regulating the growth of higher plants. Bottomley (1920) and others observed that nitrogen fixing bacteria are able to elaborate substances from carbohydrate and elementary nitrogen which promote the growth of Lemna plants. More recently, it has been found that small amounts of barnyard manure enhance growth of crop plants in some other way than can be explained merely by the content of nutrient elements and gross organic matter (Hartley and Greenwood, 1933). In view of the evidence now available, it seems probable that auxins, as well as vitamins (see Williams and Spies, 1938), produced by microorganisms in nature may have considerable influence upon growth

Up to the time when the present experiments were undertaken, it had not been shown whether or not bacteria are able to form growth substances (active on Avena) when cultured on various single amino acids or on inorganic nitrogen. The experiments reported herewith show that sterile media containing single amino acids, KNO3, or NH4Cl remain inactive (with the single exception of tryptophane) when tested on Avena after a period of 1 to 4 weeks. Tryptophane was unique in that autoclaved sterile preparations were somewhat active. (Other workers have found that the conversion of tryptophane by heat can be avoided in preparing sterile media by the use of fine filters (Georgi and

of higher plants.

Beguin, 1939), a fact which should be of practical use in future work). The data indicate further that A. aerogenes and E. coli grown in glycerol agar with single amino acids as the only source of nitrogen, and A. aerogenes grown on media supplied with inorganic nitrogen produce growth substance active on Avena. Even in the autoclaved tryptophane medium there was further elaboration of active substance by the bacteria. The presence of glycerol undoubtedly plays an important part in the synthetic processes of these organisms, though the precise relationship of an extra carbon source in the metabolism of amino acids and synthesis of growth substance is not known. In reviewing the literature, one finds considerable emphasis placed upon conversion of tryptophane to growth substance (sometimes called heteroauxin). The results reported here indicate clearly that at least some bacteria are able to form growth substance on media having no added tryptophane.

The medium containing 1/1,000 of tryptophane, in which bacteria had grown, brought about growth curvatures toward the site of application of agar on the Avena coleoptiles, while dilutions of this culture (e.g., 1/100,000) elicited curvatures away from the agar blocks. In seeking to explain the remarkably strong action of tryptophane cultures, involving both negative and positive curvatures, it should be remembered that by oxidative deamination this amino acid can be readily converted to indole fatty acids which are powerful growth substances (see Went and Thimann, 1937; Gilman, 1938). The cross transfer and growth stimulation of unilaterally applied synthetic substances in the Avena test technique have been described earlier (du Buy and Nuernbergk, 1930; Avery, Burkholder, and Creighton, 1937), and it would seem that some such explanation may be applicable here. It is known that large doses of this class of substances may cause inhibition of growth in coleoptiles (Bonner, 1933) and in buds (Thimann, 1937). Even a small amount of externally applied auxin inhibits growth in roots, but a still smaller quantity (0.2 gamma per liter) may stimulate growth in at least some roots (see Thimann and Bonner, 1938). It is possible to take the position that curvatures toward the blocks were brought about by inhibiting action of the substance at high concentrations under the blocks, and that by lateral transport small but sufficient quantities were moved into the opposite side to actually stimulate growth there. These positive curvatures were of course much greater than those which are known to occur with pure agar blocks unilaterally applied, where regenerated auxin reaches a higher concentration on the opposite side from the block and promotes a positive curvature of about 2.5 degrees (see Went and Thimann, 1937). The report of Stewart, Bergren, and Redeman (1939) concerning the occurrence of a growth inhibitor in radish cotyledons, which when applied to Avena causes positive curvatures, is of considerable interest in this connection. The work

of Goodwin (1939) also indicates that inhibiting substances may have an appreciable influence on Avena curvature. It still remains to be demonstrated whether bacteria cultured on tryptophane actually produce special inhibiting substances. Tests with the straight growth technique might throw more light on this question.

The extent to which amino acid may have been activated by the bacteria is worth consideration, on the assumption that some sort of direct conversion actually occurred. The maximum activity found with the various media amounted to about 14 to 16 degrees curvature. Curvatures of approximately this amount have been observed under the same test conditions with agar blocks containing synthetic indole acetic acid at a concentration of 156 gamma per liter. If it is assumed that the activity and molecular weight of the substances produced in bacterial cultures is of the same order as that of this well-known substance, then it might follow that about 156 gamma of activated substance per liter were present in the bacterial agar when tested. The amount found at the time of testing may not represent the total converted during the growth period, and it is indeed probable that considerable quantities of active material formed during the growth period are rendered inactive upon prolonged standing with the complicated mixture of substances in the medium. Keeping in mind the obvious limitations upon the correctness of our assumptions, it might still be possible to make a theoretical comparison of the number of molecules of active substance found in relation to the quantity of amino acid supplied in the substrate. Calculations on this question lead to the conclusion that in these particular cultures only small fractions of the supplied amino acids were converted into growth promoting substance. It is not known what the quantitative yield of growth substance may be in relation to the supply of amino acids; such calculations might be made from results of experiments with media containing much smaller quantities of amino acids than those employed in this work.

Theoretical discussion is actually of little value at this point; much additional work is needed to give anything like a satisfying explanation of the problem. Further investigation might even disclose the elaboration of non-nitrogenous growth substances of the nature of auxin a or b (see Boysen-Jensen, 1936), derived from carbohydrate (or glycerol) metabolism. Since conversion of tryptophane to active compounds, such as indole acetic acid and indole propionic acid (see Gilman, 1938), is well known, it seems natural to attribute formation of growth substances in the cultures containing this amino acid to bacterial action upon it.

In the absence of contrary evidence, it might be assumed that the various other amino acids are in some way converted to active substances. Supporting this view are the findings of Boysen-Jensen (1931b) that Aspergillus failed to produce growth substance on media containing nitrate or ammonia

as a source of nitrogen even though growth did take place. In view of the wide variation in structure of the nitrogenous compounds employed in this work, it seems reasonable to postulate formation by the bacteria of more than one kind of growth regulator, perhaps substances altogether different from the active indole fatty acids. Certainly the observation that growth substance is produced in ammonium or nitrate agar would indicate something of the remarkable synthetic powers of these microorganisms. It may be pointed out here that green plants also synthesize auxins out of organic materials made originally from inorganic compounds.

The very extensive chemical powers of microorganisms have oftentimes suggested questions concerning the possible benefits which may be derived from the end products of their own metabolism. Are growth substances, such as the auxins, of any value to bacteria and fungi? In recent years this question the negative (see Boysen-Jensen, 1936; Went and concerning auxins usually has been answered in Thimann, 1937), if answered at all.

A current paper by Ball (1938), however, indicates that 3-indole acetic acid added in amounts of 1 to 10 parts in 10<sup>7</sup> parts of synthetic medium more than doubled the rate of growth of Escherichia coli in liquid cultures. Since tryptophane was present in the culture medium used by Ball, one might inquire how the addition of small amounts of 3-indole acetic acid would be beneficial. It was concluded that "heteroauxin . . . stimulates the cells to further growth and division which produces more cells and more heteroauxin." There may be some sort of analogy here akin to the findings of West and Wilson (1939) that growth of Rhizobium trifolii cultures is remarkably stimulated in the first few hours by small additions of thiamin and flavin; then during subsequent growth, the bacteria produce ample supplies of these vitamins for their use.

It seems clear both from earlier work and in the light of the present report that microorganisms in nature are continually converting organic substrate, in some small part at least, to special substances capable of exerting regulatory action in the growth of higher plants. Certain members of this general class of growth substances, under appropriate circumstances, are known to be capable of penetrating the roots of green plants grown in soil (Hitchcock and Zimmerman, 1935; Loehwing and Bauguess, 1936; Greenfield, 1937). It has been found that these substances, which enter and move about in

plants, stimulate growth of the plant body and evoke many other remarkable responses. It seems reasonable, therefore, to suppose that traces of growth substances produced in agricultural soils or in the decomposition of farm manures used as fertilizers may exert considerable influence on the growth of field and garden crops. Extension of this line of thought would lead into problems concerning the activity of microorganisms in many different directions-e.g., in the usefulness of organic fertilizers; the action of mycorrhiza and nodule bacteria, effects of the crown gall organism and other parasites causing pathological hypertrophy, etc. Speculation is of little real value, however, without facts. It may be said that concerning the formation of growth substances by microorganisms there exists already sufficient knowledge to indicate fruitful rewards from further investigations into the mechanism of their elaboration and their rôle in the growth and development of plants.

#### SUMMARY

Avena coleoptile tests for growth promoting substances were made on cultures of Aerobacter aerogenes and Escherichia coli grown on glycerol-mineral salts-agar in which the sole source of nitrogen was either an amino acid, KNO<sub>3</sub>, or NH<sub>4</sub>Cl.

Substances producing coleoptile growth curvatures were found in the cultures supplied with any one of 17 different amino acids. Furthermore, A. aerogenes formed active substance in synthetic media containing either KNO<sub>3</sub> or NH<sub>4</sub>Cl. Uninoculated media remained inactive, with the exception of tryptophane agar which showed some activity following sterilization in the autoclave.

Although growth promotion was observed with all the inoculated substrata in certain ranges of concentration, bacterial cultures supplied with tryptophane gave positive *Avena* curvatures (toward the blocks) at higher concentrations of the activated medium, indicating growth inhibition.

Since it has been shown that bacteria elaborate growth substances in media containing any one of widely different nitrogenous compounds, it is probable that different substances having the physiological properties of auxins may be synthesized by microorganisms in ways other than by the well-known conversion of tryptophane.

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# REGENERATION OF ROOTS FROM TRANSPLANTED COTYLEDONS OF ALFALFA, MEDICAGO SATIVA<sup>1</sup>

Orville T. Wilson

THE WRITER has called attention to the readiness with which cuttings of alfalfa regenerate roots (Wilson, 1915). La Rue (1933) listed forty-two

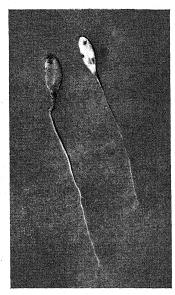


Fig. 1. Rooted cotyledons of alfalfa, Medicago sativa L. ×1.25. Photograph by Arthur Blickle.

species of plants of which he tested the regeneration of roots from cotyledons by transferring the removed cotyledons to moist filter paper in Petri dishes and noting development. Of the forty-two

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species, *Medicago sativa L*. was the only one which failed to regenerate roots. As this result was contradictory to the writer's experience with alfalfa, additional experiments were carried out to test the regenerating capacity of the cotyledons.

In these experiments the cotyledons were removed from seedlings in the early juvenile leaf stage and set, cut end down, in moist greenhouse soil, with most of the blade exposed. A glass cover was placed over them to prevent excessive evaporation, but this was removed after the first week. After three weeks, approximately 50 per cent of the cotyledons were green and healthy (80 per cent in one experiment). When these were removed from the soil, they were found to be well rooted in a majority of cases. In one experiment sixty-seven cotyledons out of one hundred transplanted, regenerated roots. Two rooted cotyledons are shown in figure 1.

For an adequate discussion of the problem of root regeneration from cotyledons the reader is referred to La Rue's paper (1933). The present record serves only to supplement the long list of positive results there presented.

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# THE FLORAL ANATOMY OF THE AVOCADO 1

Philip C. Reece

This study of the anatomy of avocado flowers is a part of an investigation now under way on the family Lauraceae. This article is to be followed by a report on fruit bud formation of avocados and by a morphological interpretation of the apparent evolutionary tendencies in the Lauraceae, based upon comparative data.

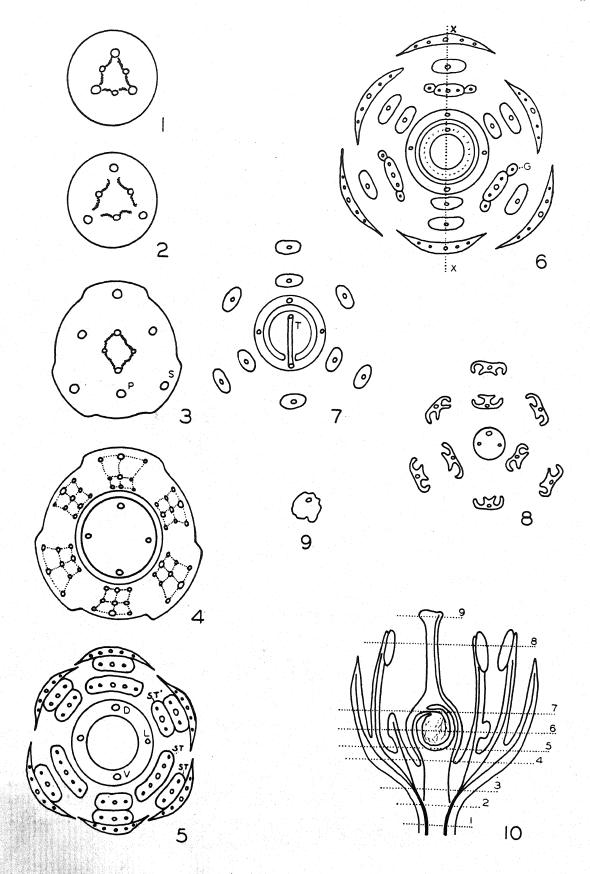
The family inhabits the tropical regions of the Americas, Asia, Australia, and Polynesia. The avocado is native to Central America and Mexico. Its culture has now become a thriving industry in the relatively frost-free areas of California and Florida.

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The writer wishes to express his appreciation to Dr. A. J. Eames, who, by his advice and criticism, aided in the development of this manuscript. Part of the research here reported was carried on in the laboratory of the Department of Botany, Cornell University, Ithaca, New York.

All the cultivated varieties of avocados belong to the single species, *Persea americana* Mill. (*Persea gratissima* Gaertn.)

The regular, bisexual, trimerous flowers of Persea are borne in cymose panicles. The floral plan of three, so conspicuous in this species, is popularly associated with monocotyledonous angiosperms. However, this fundamental plan of three is more prevalent among dicotyledons than is generally realized. The yellow or greenish six-parted perianth consists of two whorls upon which are inserted four stamen whorls of three members each. The anthers of the two outer whorls are introrse. Those of the third whorl are extrorse and bear glands on each side of the filament near the point of attachment on the perianth. The fourth whorl has been reduced to gland-like staminodes. The uni-



locular ovary is superior. The simple style is terminated by a small obtuse stigma. The solitary, pendulous, anatropous ovule ripens to produce a very large seed which is devoid of endosperm. The seed coat is membranous.

Examination of sections of the pedicel reveals the vascular tissue arranged in a siphonostele. Six areas of primary xylem stand out sharply, with the tissue in the intervening regions in a procambial state (fig. 1). At higher levels, approaching the receptacle, these areas depart from the stele and pass through the cortex as six tepal traces. (The term tepal is used in this report to designate any perianth segment, regardless of whether it is a member of the inner or outer whorl. At this point, no attempt is made to identify the segments as sepals or petals.) Three traces advance slightly ahead of the others and supply the outer perianth whorl (fig. 2).

After the departure of the tepal traces, the gaps left in the stele quickly close. Only a limited amount of vascular tissue remains. The stele "shrinks" toward the center of the axis, as the end of the receptacle is approached (fig. 3). The carpellary traces depart near the tip of the axis as the stele "closes in." The carpellary supply consists of a dorsal trace (midrib), two or more lateral traces, and two ventral traces (marginal veins) which are united into a single, morphologically compound vascular bundle. These traces enter the ovary (fig. 4) with the dorsal trace lying along the radius of a member of the outer perianth whorl. The ventral trace stands directly opposite the dorsal or at an angle of 180°.

The members of each perianth whorl are coherent at the base, as well as adnate to each adjacent whorl. A cup is thus formed around the base of the ovary by the perianth with the stamens perigynously inserted upon it. In this cup, the tepal traces divide, indicating that at lower levels the bundles are morphologically compound. The branching of the bundles is in a tangential plane, so that a tepal trace and two stamen traces lie upon the same radius. This is immediately followed by branching in the radial plane. Traces apparently form a reticulate vein system with anastomosing connections between the vascular supplies of the various whorls (fig. 4).

All the stamens have three traces traversing the major portion of the filament. The lateral veins in each filament weaken and disappear below the anthers. Each stamen of the third whorl possesses a gland on each side of the filament near the base. The glands are supplied by two additional veins which arise by the radial forking of the outer veins in the filament base. Then five traces are in a row (fig. 5). The veins enter the glands above the level at which the members of all the whorls become free (fig. 6).

The single, large anatropous ovule is attached to the parietal placenta near the top of the loculus. A bundle, which has been formed by the fused ventrals, enters the funiculus of the ovule and continues down the opposite side through the raphe (fig. 7). The bundle in the ovule branches profusely, so that the integument is traversed by a network of veins. These are conspicuously developed in the seed coat when the fruit is mature.

The fourth androecium whorl consists of three staminodes, which are not as high as the ovary. The anthers of the stamens of the two outer whorls are introrse, those of the third whorl are extrorse, and are borne on long filaments which extend to about twice the height of the ovary. The dorsal and two lateral carpellary traces extend up through the simple style (fig. 8). The lateral traces disappear below the stigma. The strong dorsal trace extends into the stigma (fig. 9).

It is becoming generally recognized that imperfect or incomplete flowers have attained that condition through simplification and loss of parts. They are simple through reduction and not primitively simple. Anatomical evidence of such simplification persists long after external evidence has disappeared. Therefore anatomical investigation of the floral anatomy supplies valuable criteria for a morphological interpretation of the flower.

Considerable difference of opinion exists regarding the nature of the perianth in this genus. Small (1913) believes that the perianth consists of six deciduous sepals, in two series, united at the base. Popenoe (1914) refers to the perianth as a "calyx deeply six-parted,—the corolla wanting." Gray

Fig. 1-10. Persea americana Mill. In these figures: S, sepal (outer tepal) midrib; P, petal (inner tepal) midrib; ST, stamen bundle; ST', staminode bundle; G, gland; D, dorsal carpellary trace; V, ventral carpellary trace; L, fateral carpellary trace; T, ovule trace.—Fig. 1-9. Cross section diagrams of the flower.—Fig. 1. Through the pedicel.— Fig. 2, 3. Successive levels showing the departure of the outer tepal (sepal) traces and the inner tepal (petal) traces. The carpel dorsal (midrib), the fused ventrals (marginal veins) and two median lateral veins are distinguishable at the level of fig. 3. The vascular tissue of the stele is fading out at the end of the axis.—Fig. 4. The perianth cup surrounds the base of the ovary. The sepal and petal traces have divided tangentially to produce the traces of four stamen whorls and radially to produce branch traces in each whorl.—Fig. 5. The perianth segments and the filaments of the third stamen whorl have become free. The locule is visible in the ovary.—Fig. 6. All floral whorls are free. The longitudinal section in fig. 10 is in the plane X-X. The ovule trace, derived from the fused ventrals, enters the funiculus and passes down the opposite side of the ovule near the dorsal. The integument is invested with numerous small branches of this trace.—Fig. 7. Section through the top of the ovule showing the origin of the ovule trace. The lateral stamen traces have disappeared in the upper portion of the filaments. The fourth stamen whorl reduced to staminoides has disappeared between sections indicated by fig. 6 and 7. The perianth has been omitted from this diagram.—Fig. 8. Through the anthers and style.—Fig. 9. The dorsal trace continues to the stigma-Fig. 10. Longitudinal diagram of the flower. The levels of the cross section diagrams fig. 1 to 9 are indicated. The longitudinal diagram is in the plane X-X in the cross section fig. 6.

(1908) and Hutchinson (1926) also speak of the six-parted calvx and imply the lack of a corolla. This viewpoint may be attributed to a lack of anatomical evidence and to hesitancy in interpreting the perianth of a dicotyledon as consisting of a three-parted calvx and a three-parted corolla. However, Van Tieghem (1891) considered the floral formula to be "(3 S + 3 P + 3 E + 3 E" + 3 E" + 3 E") + C" and said that "the calvx and the corolla, composed each of a single whorl, are sepaloid or petaloid, but like each other and concrescent in a tube. The median sepal is posterior."

The floral anatomy supports the view of Van Tieghem. There is no evidence that the corolla is absent. The concrescence of the perianth into a tube and the resemblance of one whorl to the other may not be accepted as valid criteria for considering that the perianth consists of a calyx only. If such reasons were to be considered valid, they should be applied in the interpretation of the perianth in liliaceous forms, in which the morphological conditions of the calyx and corolla are exactly the equivalent of those found in the perianth of the avocado. However, the fact that the perianth traces arise at two levels is in itself sufficient evidence that the perianth does not consist of a calyx alone.

Comparative studies of present plant forms as well as paleobotanical studies indicate that the fundamental type of architecture in pteropsid vascular plants is dichotomy. Working upon this theory, Hunt (1937) investigated the vascular anatomy of the style and stigma in certain groups. A study of the gross and anatomical structure of these organs convinced him that evidence still exists in the modern flower indicating that the carpel has been ultimately derived by reduction from a dichotomous branch system through the intermediate stage of an unspecialized palmately three-lobed appendage. The simple stamen commonly found in angiosperms may also have been similarly derived by reduction from a sporophyll which, in the ancestors of the angiosperms, was probably a fertile branch system.

The condition in which the vascular supply to a number of stamens arises as the result of the division of a single bundle has been investigated in a number of forms by Wilson (1937), who believes that such stamens have been derived from a primitive branch system, the ultimate branchlets of which were terminated by sporangia.

Reduction in most angiosperms has progressed so far in the stamen that only a single trace passes through the filament to the anther which consists of two bi-sporangiate synangia brought into close conjunction. It may therefore be assumed that the additional traces in an avocado stamen probably indicate a retention of a primitive condition. The various whorls exhibit various degrees of reduction. Members of the third whorl are the most primitive. Although each trace is fused with a tepal trace through part of its course, the trace later branches to produce three bundles, a median which extends to the anther and two laterals which pass some dis-

tance through the parenchyma of the somewhat fleshy stamen. The lateral bundles branch dichotomously near the base of the filament to supply the glands. Reduction of a primitive fertile branch system has probably proceeded in many directions to produce the stamen. The avocado stamen may have been derived from a much branched structure through some intermediate three-branched stage, forming a series similar to the series postulated by Hunt for the carpel.

The gynoecium consists of a single carpel. The existing carpel usually lies in the plane of a sepal (outer tepal) and is alternate with the members of the fourth androecium whorl (staminodes). However, the carpel frequently lies on the radius of a petal (inner tepal) and opposite a staminode. The writer believes that this is evidence of reduction from a multi-carpellate ancestor.

The great increase in size which comes with the ripening of the ovary is accompanied by the formation of many additional cells in the pericarp. In the mature fruit, although the main vascular system remains fundamentally the same, it is complicated by branches which are differentiated in the new tissue. These branches consist chiefly of protoxylem cells accompanied by a few elongated parenchyma cells.

The presence of a single seed with its very large embryo and no endosperm indicates a high degree of specialization. Indeed, the gynoecium has become so specialized that in so far as this species is concerned all evidence indicating the presence of more than one ovule in ancestral forms is lost. The profuse vascular supply in the integument may be regarded as the retention of a primitive characteristic. This condition has been retained coincident with the development of the large seed.

Hutchinson (1926) states that the members of the Lauraceae represent the extreme limits of reduction in the Magnolian alliance and that valvular dehiscence in this group and in the Berberidaceae is probably due to parallel evolution and is not a sign of affinity. He places them in the Apetalae but upon a different line from that giving rise to the Amentiferae.

The writer has investigated the floral anatomy of the Fagaceae (1938) and finds striking similarities of floral and inflorescence characters. Both groups are mainly tropical and display a trimerous floral plan with a six-parted perianth and twelve perigynously inserted stamens. The gynoecium in each case has apparently been reduced from a six-carpellate ancestor. A network of veins in the membranous seed coat surrounds the large fleshy embryo. The ament of the Fagaceae is also fundamentally a cymose panicle but is much more specialized than that of the Lauraceae.

The possibility of a common origin of the Lauraceae and Fagaceae in the early differentiation of Angiosperms is suggested by these similarities of floral and inflorescence characters. However, these similarities are the result of parallel evolution and do not indicate close relationship.

#### SUMMARY

This study of the floral anatomy of *Persea americana* reveals that this species is clearly a specialized form derived from a primitive angiosperm stock and that the perianth consists of a three-parted calyx and a three-parted corolla. The anatomy does not support the interpretation that the perianth consists of a six-parted calyx or that the corolla is absent.

The vascular anatomy of the stamen indicates that it has been derived by reduction from a branch system. Vertical compression has resulted in the fusion of the vascular supply of the main limb or rachis of this branch system to the bundles supplying the perianth.

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# MORPHOLOGY OF TINOSPORA CORDIFOLIA, WITH SOME OBSERVATIONS ON THE ORIGIN OF THE SINGLE INTEGUMENT, NATURE OF SYNERGIDAE, AND AFFINITIES OF THE MENISPERMACEAE <sup>1</sup>

#### A. C. Joshi

As LITTLE work had been done on the embryology and cytology of the family Menispermaceae prior to 1933, an investigation of some members of this group was started by the writer in that year. During the course of this work an account of the development of pollen was published (Joshi and Rao, 1935), and in 1937 the writer described the structure of the gynoecium and development of the ovule and embryo-sac in Cocculus villosus. In the present paper a similar study has been made of Tinospora cordifolia Miers.

The earlier work on the family was fully summarized in the paper on Cocculus villosus (Joshi, 1937). There is therefore no necessity to recapitulate it here. It is necessary only to mention that since then the writer has come across a small embryological work on Tinospora cordifolia by Abraham (1935), but this is not very critical, and in it several features have been left unobserved by the author.

THE DEVELOPMENT AND STRUCTURE OF THE OVULE.—The gynoecium of Tinospora cordifolia consists of 3-6 free carpels. The most common number is three. Gynoecia with four, five, or six carpels are increasingly rare. These carpels arise from the floral axis in a spiral order, and when the number is more than three, the last carpel is frequently abortive just as in Cocculus villosus (Joshi, 1937).

The mature carpels are always uni-ovulate (fig. 3), but, as in *Cocculus*, two ovules are present in each carpel in the early stages (fig. 1). One ovule <sup>1</sup> Received for publication March 22, 1939.

arises from each of the two margins of the carpels. As the carpellary margins come close together and fuse, the two ovules come to lie nearly in one line, and one of them takes up a position slightly above the other. Both continue to develop equally until the megaspore-mother cell stage (fig. 1). Generally after this, growth suddenly stops in the lower ovule, which is then gradually crushed into an insignificant scaly structure by the developing upper ovule (fig. 2, 3). One can see the beginning of this gradual degeneration of the lower ovule as the tetrad is formed in the upper. By the time the latter reaches the 4-nucleate embryo-sac stage (fig. 2), the lower has completely lost its shape.

This behavior of the ovules in Tinospora, however, is not so constant as in Cocculus. While examining the latter (Joshi, 1937), I found no exception to this condition. In Tinospora, exceptional cases are frequently seen. Figure 5 shows a carpel in which both the ovules have reached the 4-nucleate embryo-sac stage simultaneously. Figure 4 shows a carpel in which the lower ovule has reached the 4-nucleate embryo-sac stage, while the upper is still at the one-nucleate embryo-sac stage. But such variations are of no importance in the end. By the time the mature embryo-sac is formed in the upper ovule, the lower ovule always ceases to grow and is crushed in every case. The general form and structure of the carpel at this stage is shown by figure 3, and no exception to this condition has been observed.

Both ovules after their differentiation from the carpellary margins continue to grow straight till

they meet the dorsal wall of the carpel (fig. 1). Their form up to this stage is perfectly orthotropous. After meeting the opposing carpel wall, the upper ovule turns upward and the lower downward. The functional ovule finally assumes an amphitropous form (fig. 2, 3) under the same influences as act in Cocculus villosus (Joshi, 1937). A small difference is seen only in the post-fertilization stages. In Cocculus, when endosperm develops, there is a marked increase in the size of the funicle, and the ovule becomes campylotropous. In Tinospora, there is no such development of the funicle, and the ovule remains amphitropous throughout its life, though it becomes furrowed on the ventral side due to the development of a longitudinal ridge on the ventral suture of the carpel. This can be seen in transverse sections of a carpel.

The ovule of *Tinospora cordifolia* differs from that of *Cocculus villosus* in the number of integuments. In *Cocculus* (Joshi, 1937), there are two integuments, but there is only one in *Tinospora*. The single integument of *Tinospora*, however, is more massive. In *Cocculus*, both the integuments for their greater length are two to three cells thick. In

Tinospora, the single integument is three cells thick in the micropylar region, but it thickens towards the chalazal end and is here five to six, and for a short distance even more, cells thick. Another point to be noted in the structure of the integument of Tinospora cordifolia is the sudden attenuation in its thickness (generally from five to three cells) on the ventral side just as it becomes free from the nucellus (fig. 2, 3, 7). There is generally a small notch visible here. The difference seen between the structure of the integuments of Tinospora and Cocculus in the mature condition is also present in their initials. This is brought out by comparison of figures 6 and 10. The two initials of the integuments of Cocculus are comparatively slender (fig. 10), while the single integumentary initial of Tinospora is comparatively massive (fig. 6).

The form of the micropyle in *Tinospora cordifolia* is not constant, as may be seen from figures 2 and 3. It may be nearly straight (fig. 2) or, due to greater development of the integument from the dorsal as compared with the ventral side, zig-zag (fig. 3). Between the integument and the nucellus in

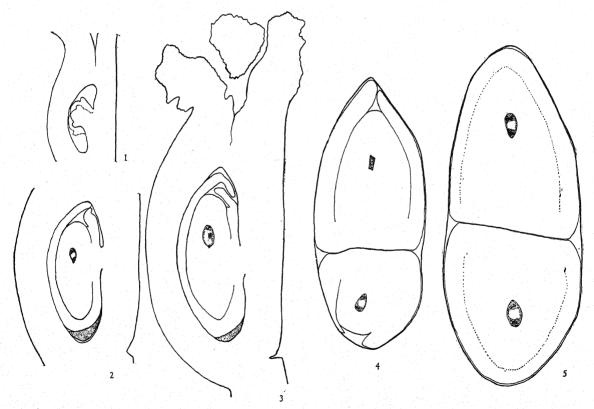


Fig. 1-5.—Fig. 1-3. Tinospora cordifolia. Longitudinal sections of carpels at various stages of development. Fig. 1 shows a carpel with two equally developed ovules at the megaspore-mother cell stage. Fig. 2 shows the lower ovule crushed and the upper at the 4-nucleate embryo-sac stage. Fig. 3 shows the upper ovule at the mature embryo-sac stage, while the lower is still further reduced. ×169.—Fig. 4-5. Tinospora cordifolia. Portions of longitudinal sections of two carpels in which the lower ovule is exceptionally developed. Only the parts to the inside of the inner boundary of the carpel are shown. In fig. 4 the lower ovule shows a 4-nucleate embryo-sac, while the upper is still at the 1-nucleate embryo-sac stage. In fig. 5 both the ovules are at the 4-nucleate embryo-sac stage. Where the boundary between the integument and the nucellus is indistinct, it is shown by dotted lines. ×359.

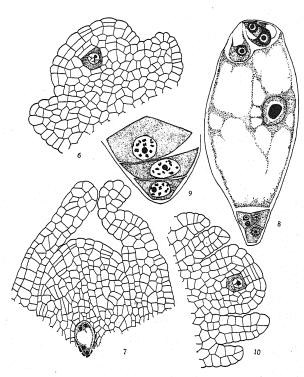


Fig. 6-10.—Fig. 6-9. Tinospora cordifolia.—Fig. 6. One of the ovules shown in fig. 1 at a higher magnification showing cellular structure.—Fig. 7. Micropylar part of the ovule shown in fig. 2 at a higher magnification and showing cellular structure.—Fig. 8. A mature embryosac showing besides the normal features an egg-like synergid and a 2-nucleate antipodal.—Fig. 9. Antipodals from a mature embryo-sac at a higher magnification showing their nuclei in prophase.—Fig. 10. Cocculus villosus. Part of a longitudinal section of an ovule at the megaspore-mother cell stage showing the structure of the nucellus and integuments. Fig. 6, ×910; fig. 7, ×546; fig. 8, ×1740; fig. 9, ×3520; fig. 10, ×910.

the micropylar region of the ovule there is generally present a small air-space (fig. 2, 3, 7).

Another point about the ovule of Tinospora cordifolia which seems to be of some morphological importance is its spatial relations with the inside of the carpel. Going through the series of sections, one always sees in Tinospora the closeness with which the ovule fits inside the ovary. Air-spaces are nearly absent. Only a small one is seen near the micropyle of the ovule (fig. 3), and in the early stages another may be present at the opposite end (fig. 2). At other points the carpel and the ovule so press each other that even the boundaries between them are not very distinct. In Cocculus, there are airspaces between the two integuments and between the inner integument and the nucellus (fig. 13). There are no such air-spaces in Tinospora. This closely pressed condition of the ovule inside the carpel in Tinospora is still further emphasized in carpels where two ovules continue to develop equally even after the megaspore-mother cell stage. In such cases even the small air-space that is seen in the

micropylar region of the ovules tends to disappear (fig. 4) or disappears completely (fig. 5), and the boundary between the integument and the nucellus tends to become partially or completely indistinct. There is thus no clear line in figure 5 between the integument and the nucellus. It is therefore shown in the figure only by dots.

As the spatial relations of the ovule and the carpel in *Tinospora* are of considerable importance in indicating the cause of the origin of the single integument and naked (integument-less) ovules, photomicrographs of sections from which figures 3 and 5 were drawn are presented in figures 11 and 12. For the sake of comparison, a similar photomicrograph of a carpel of *Cocculus villosus* is shown in figure 13. These clearly demonstrate the points described above.

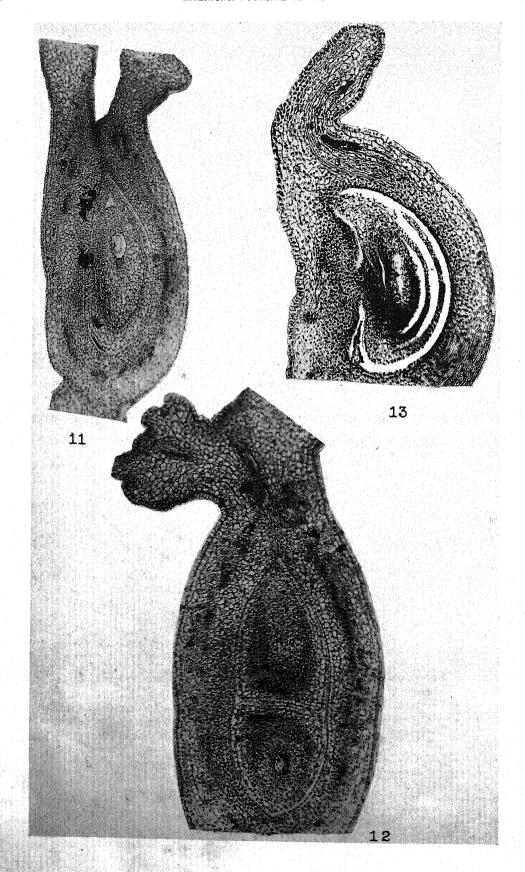
The structure of the nucellus in *Tinospora* is similar to that of *Cocculus* (Joshi, 1937), except that it is slightly better developed. There is a similar cap formation by periclinal divisions in the epidermis (fig. 6, 7). The parietal tissue is more developed than in *Cocculus*. Consequently the embryo-sac in *Tinospora* is deep-seated, and about 15 layers of cells cover it (fig. 7).

THE DEVELOPMENT AND STRUCTURE OF THE EMBRYO-SAC.—The development of the embryo-sac in Tinospora cordifolia is similar to that of Cocculus villosus (Joshi, 1937) and corresponds to the normal type. There is a single hypodermal archesporial cell, which cuts off a primary wall cell. The megaspore-mother cell gives rise to a linear or occasionally T-shaped tetrad of megaspores out of which the chalazal megaspore develops into the 8-nucleate embryo-sac by three free nuclear divisions.

The mature embryo-sac is about  $100 \mu$  long, as in Cocculus, but is slightly different in shape, being broader in the middle (fig. 8). Only two points in its structure require notice here. The synergidae in most cases are quite normal in structure, but occasionally they are egg-like (fig. 8, upper synergid), showing cytoplasm and nucleus at the chalazal end and the large vacuole toward the micropylar end.

The second point refers to the antipodals. The nuclei of the antipodals as these cells are organized are in the resting condition, and they show this condition in young 8-nucleate embryo-sacs. After the polar nuclei have fused, the nuclei of the antipodals prepare to divide. They enter the prophase stage (fig. 9), but generally the division is not completed, and they remain in this condition until the disorganization of antipodals. In a few cases, however, these nuclei have been seen to divide completely, and some antipodal cells become 2-nucleate (fig. 8, the left hand upper antipodal).

Discussion.—Origin of the single integument.— The embryological characters of Tinospora cordifolia closely resembles those of Cocculus villosus described previously (Joshi, 1937) except in the form of the ovule and the number of integuments. In Cocculus villosus the ovules are amphitropous up to the



embryo-sac stage but become campylotropous afterward. In Tinospora cordifolia they are amphitropous throughout their life. The number of integuments is two in Cocculus villosus, while the ovules of Tinospora cordifolia possess only one integument. In showing this difference between closely related plants, the family Menispermaceae agrees with families like Ranunculaceae, Rosaceae, and Leguminosae (Schnarf, 1931). Such groups are interesting morphologically, because from comparison of closely related forms some light can be thrown on the origin of the single integument.

Because the primitive Archichlamydeous families among the Dicotyledons mostly show two integuments and the single integument of the Metachlamydeous Dicotyledons is in general as massive or even more massive than both the integuments when there are two, Coulter and Chamberlain (1903) long ago expressed the opinion that the single integument "represents two integuments in the sense that their primordia are no longer differentiated." This generally accepted view needs a little modification in the case of Tinospora cordifolia. Comparison of this species with Cocculus villosus shows that the single integument of the former in the chalazal part is nearly as massive as both the integuments of the latter put together. A similar difference is seen in the primordia of the integuments in the two genera. There is therefore no doubt that in the chalazal part of the ovule the single integument of Tinospora has resulted from the fusion of two integuments like those of Cocculus, but a different interpretation is necessary for the micropylar part of the integument. In this region the integument is only as thick as the single integument of Cocculus. Further, just as it separates from the nucellus, it shows a small notch on the ventral side. At this point it undergoes a somewhat sudden decrease in thickness, and it appears as if the former inner integument is coming to an end. For these reasons the single integument of Tinospora in the micropylar part of the ovule is equivalent only to the outer integument of Cocculus.

Very little is known as present about the probable cause of the origin of the single integument from two. On this point also a comparison of the carpels and ovules of Tinospora and Cocculus throws some light. Such comparison shows that there is only one difference between the two plants that can account for the replacement of two integuments by one, and that is the larger size of the ovule as compared with the ovary in Tinospora. This leads to the closely pressed condition of the ovule inside the carpel and the obliteration of most of the air-spaces. It can be reasonably suggested that the mechanical pressure which is thus exerted on the ovule during its development may have been the primary cause of the replacement of two integuments by

one, by selecting variations in this direction. A careful comparison of other closely related plants, such as species of *Delphinium* and other Ranunculaceae, which are known to differ in the number of integuments should throw more light on this subject.

An extension of the above hypothesis can also indicate the method by which naked (integumentless) ovules, which are found in a few flowering plants,<sup>2</sup> could have originated in some cases. We see in Tinospora, when two ovules in a carpel continue to develop equally even after the megaspore-mother cell stage, the boundaries between the different parts tend to become indistinct as a result of the extra mechanical pressure to which they are subjected. There is thus in figures 5 and 12 no clear line between the nucellus and the integument. The integument can be distinguished only by the different form of its cells. If these cells were similar to those of the nucellus, no difference would be visible between the two, and the ovule would be described as naked. The naked ovules such as have been described in the saprophytic Gentianaceae Leiphaimos and Cotylanthera by Oehler (1927) appear to have developed in this manner. This is clear from the orientation of their embryo-sac.3

Morphology of the synergidae and embryo-sac.— The synergidae differ from the egg cell in their vacuolation and the position of cytoplasm and nucleus. The egg has a large vacuole toward the micropylar end, while the cytoplasm and the nucleus are pressed toward the chalazal end. In the synergidae the position of these parts is just the reverse. The vacuole is found toward the chalazal end, while the nucleus and cytoplasm occupy the micropylar end.

<sup>2</sup> According to Wettstein (1935), naked ovules occur in Santalaceae, Loranthaceae, Balanophoraceae, Olacaceae, saprophytic Gentianaceae, and species of *Crinum*. To this list may be added the genus *Houstonia* of the Rubiaceae (Lloyd, 1902).

3 Maheshwari (1937) in his recent review of the embryo-sac of angiosperms, while dealing with the reversed polarity of the embryo-sac, remarks that the saprophytic Gentianaceae like Leiphaimos and Cotylanthera, the Loranthaceae, and the Balanophoraceae provide the best instances of reversed polarity of the embryo-sac. This would be so if the ovules of these plants were really orthotropous. In these saprophytic Gentianaceae, however, it is clear that they are not so. They are only apparently orthotropous. Actually they are anatropous, and the embryosac is quite normally orientated. The families Loranthaceae and Balanophoraceae are highly specialized, and the morphology of the ovule in these families is not very clear. To describe their embryo-sac as reversely orientated in the light of our present knowledge is unjustified. In dealing with the reversed polarity of the embryo-sac, it is better to leave such examples alone. Perhaps it would be better to interpret the morphology of the ovule in these families by a study of the orientation of the embryo-sac. In the writer's opinion, complete reversed polarity of the embryo-sac is not the normal character of any angiosperm. It always occurs as a rare abnormality, and the case of Woodfordia floribunda described by Joshi and Venkateswarlu (1935) is a good illustration of this.

Fig. 11-13.—Fig. 11. Tinospora cordifolia. Longitudinal section of a normal carpel with the ovule at the mature embryo-sac stage.  $\times 60$ .—Fig. 12. Tinospora cordifolia. Longitudinal section of a carpel with two ovules showing 4-nucleate embryo-sacs.  $\times 90$ .—Fig. 13. Cocculus villosus. Longitudinal section of a carpel with the ovule showing mature embryo-sac.  $\times 120$ .

The synergidae of Tinospora cordifolia are interesting in showing occasionally egg-like structurei.e., vacuole toward the micropylar end and cytoplasm and nucleus toward the chalazal end. Similar egg-like synergidae have been observed in Iris sibirica (Dodel, 1891), Aconitum napellus (Osterwalder, 1898), Trillium grandiflorum (Ernst, 1902), Mercurialis annua (Malte, 1910), Myricaria germanica (Frisendahl, 1912), Delphinium elatum (Persidsky, 1914), and Fritillaria pudica (Sax, 1916). They have also been described by Svensson (1925) in Nemophila atomaria, by Puri (1934-1935) in Moringa oleifera, and Johri (1936) in Sagittaria graminea. During the course of the embryological investigations carried out in this department in recent years, synergidae with egg-like structure have been found in Gisekia pharnaceoides (Joshi and Rao, 1936), Tamarix dioica (Joshi and Kajale, 1936), Boerhaavia diffusa and B. repanda (Kajale, 1938), Clerodendron Phlomidis (Misra, 1939), and several Amarantaceae and Bergia ammanioides (Kajale, unpublished). As these examples are from plants which come from different families, it is very likely that future research may reveal many more instances, since egg-like synergidae are not a rarity, as might have been supposed a few years ago.

Development of embryos from synergidae has been seen in Mimosa Denhartii (Guignard, 1881), Orchis morio (Strasburger, 1886), Iris sibirica (Dodel, 1891), Lilium martagon (Overton, 1891), Aconitum napellus (Osterwalder, 1898), Najas major (Guignard, 1901), Trillium grandiflorum (Ernst, 1902), Delphinium elatum (Persidsky, 1914), Hypericum maculatum (Schnarf, 1914), Gastrodia elata (Kusano, 1915), Fritillaria pudica (Sax, 1916), Sagittaria graminea (Johri, 1936), and Boerhaavia repanda (Kajale, 1938), and it appears that in many of these cases it is due to the fertilization of egg-like synergidae. The synergidae therefore do not differ fundamentally from the egg and should be regarded as of the same nature.

With reference to the morphology of the angiospermous embryo-sac, we find, as Maheshwari (1937) has recently summarized, three chief views. According to Porsch (1907), the 8-nucleate embryosac of angiosperms is equivalent to two archegonia without any prothallial tissue. The synergidae, egg, and the upper polar nucleus form one archegonium, -the synergidae being equivalent to neck cells and the upper polar nucleus to the ventral canal nucleus,—while the three antipodals and the lower polar nucleus form the second but now functionless archegonium. According to Schürhoff (1928), one synergid (= a ventral canal nucleus) and the egg constitute one archegonium, the second synergid and the upper polar nucleus constitute a second archegonium, while the antipodals and the lower polar nucleus are prothallial. According to the third view, all the nuclei of the embryo-sac are potential eggs, although only one of them produces an embryo. The previous observations on the na-

ture of the synergidae support the last view about the morphology of the embryo-sac. This is further supported by the occasional occurrence of egg-like antipodals and even their fertilization, as in *Ulmus americana* (Shattuck, 1905), and rarely by the reversal of the polarity of the whole embryo-sac, as in *Lawsonia inermis* (Joshi and Venkateswarlu, 1935)

Affinities of the Menispermaceae.—At present it is generally maintained that the family Menispermaceae is related to the Magnoliaceae and Ranunculaceae alliance and can be placed definitely in the order Ranales of Bentham and Hooker (1862-1883) or Engler and Gilg (1924), or Polycarpicae of Wettstein (1935). Within the Ranales or the Polycarpicae, however, there are two distinct views about its affinities. Wettstein, on account of the woody habit of its members and the occurrence of secretory cells in the parenchymatous tissue of the vegetative parts, believes that it is more closely related to the Magnoliaceae and allied woody families. Hutchinson (1926), on the other hand, although he attaches even greater importance to habit of the plants in his classification of the dicotyledons, thinks that the Menispermaceae are more nearly related to the Ranunculaceae. We can now discuss what light embryological evidence throws on this question.

The development of the embryo-sac both in the Ranunculaceae and Magnoliaceae is very similar, but there are a few other features which appear to be of some value for our study. In all the Magnoliaceae investigated so far the number of integuments is constantly two, while it is variable in the Ranunculaceae. The Menispermaceae thus agrees with the Ranunculaceae. The antipodals of the Menispermaceae in showing the tendency to divide resemble more those of the Ranunculaceae (Huss, 1906) than the small, often early degenerating antipodals of the Magnoliaceae (Schnarf, 1931). Finally, there is one characteristic feature of the pollen grains of Tinospora which appears to be of great significance in this connection. This is the fringed margin of the germ pore (Joshi and Rao, 1935). Pollen grains with fringed margin of the germ pores are found in the monocotyledonous families Alismataceae, Butomaceae, and Hydrocharitaceae (Wodehouse, 1936b), but among the dicotyledons they are found only in the Ranunculaceae (Wodehouse, 1936a). Although the pollen of a number of Magnoliaceae has been studied (Wodehouse, 1935), grains with fringed margin of the germ pores have not been found in any case. The evidence from the present studies, therefore, is in favor of placing the Menispermaceae with the Ranunculaceae as suggested by Hutchinson (1926).

#### SUMMARY

The gynoecium of Tinospora cordifolia consists of 3-6 spirally arranged carpels. When the num-

ber is more than three, the last carpel is frequently abortive. Two ovules differentiate at first in each carpel, but later the lower is suppressed. The functional ovule is amphitropous, has only one integument, and is closely pressed inside the carpel. The nucellus shows an epidermal cap. The development of the embryo-sac is quite normal. The synergidae frequently show egg-like structure. The nuclei of the antipodals show a tendency toward division.

The single integument appears to have resulted from the fusion of two in the chalazal and suppression of the inner in the micropylar part. The synergidae are of the same nature as the egg. The family Menispermaceae is more closely related to the Ranunculaceae than to the Magnoliaceae.

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# CAULOXYLON AMBIGUUM, GEN. ET SP. NOV., A NEW FOSSIL PLANT FROM THE REED SPRINGS FORMATION OF SOUTHWESTERN MISSOURI <sup>1</sup>

J. E. Cribbs

THE PETRIFIED stem described in this paper was found in Greene County, Missouri, and was given to E. L. Clark, professor of geology at Drury College, who referred it to the writer for identification.

It was picked up from an accumulation of chert in a small ravine, and, although not embedded when found, it is assumed to have been included in a chert matrix which fractured and broke up under the influence of weathering. Since this chert has been ascribed to the upper part of the Reed Springs Formation, the plant probably lived during the earlier half of Mississippian time.

STATE OF PRESERVATION.—The portion of stem found is about 15 cm. long, 7.5 cm. wide, and 5.5 cm. thick. It is straight and without branches or appendages other than leaves which are represented

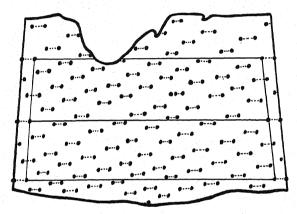


Fig. 1. A flat projection of the stem surface, showing the distribution of leaf traces as they entered the cortex. Paired traces are connected with broken lines. Three superposed nodes of the same orthostichy are shown at the left and repeated at the right.  $\times 5/14$ .

by traces in the secondary wood. The elliptical outline as seen in transverse section (fig. 18) is due to compression. All structures external to the secondary wood are lost, and the latter may not be complete. The pith, primary wood, secondary wood, and leaf trace bundles are preserved and infiltrated with siliceous materials. There is evidence that the stem was bruised and buffeted about before fossilization took place, a fate common to stems carried out to sea by rivers or derived from eroding shores. The obscure condition of pits on the walls of primary and secondary tracheids is probably due to partial decay before petrifaction. Throughout all tissues there is an extensive development of spherulites which in many places obscure the structural features and make observation difficult.

Phyllotaxis.—Regularly arranged on the surface of the stem are many small protrusions which sections prove to be convoluted knots of tracheids developed opposite the terminus of leaf trace bundles. Since these are visible on all of the surface area (fig. 19), the phyllotaxis could readily be determined.

A flat projection of the stem exterior drawn to scale with the distribution of outgoing leaf traces is shown in figure 1. The traces originate and pass out through the secondary wood in pairs. One bundle (that in the anodic position) is slightly higher than the other where it enters the secondary wood from the pith. The two bundles of a pair are shown connected by a broken line in the figure. Three successive nodes of an orthostichy are shown at the left, and the same three appear again at the right side. Between the lower and middle horizontal lines lie all the paired traces which intervene between the first and second nodes of the orthostichy. The ascending spiral is toward the right, and the thirtyfourth node lies directly over the first in series. Between the middle and upper horizontal lines there is the same arrangement. The apparent spirals toward the right are parastichies. Each successive node in the true spiral occurs in the third parastichy to the right of the preceding one, thus the spiral passes around the stem twenty times to reach the thirty-fourth node above, and the phyllotaxis may be represented by the fraction 20/34.

One irregularity to be observed in the trace arrangement is shown in the figure at the fourteenth node of the second series, where three traces emerge at the surface instead of two. All three lie on a horizontal plane. From the grouping of the traces, it is apparent that they should be interpreted as pairs which continued into the cortex with or without further division before they entered the petioles. It will be observed that the vertical spacing is different toward the two ends of the stem, being greater distally. The average distance between two successive nodes of the same orthostichy is about 4.5 cm., which gives an average internodal distance of 1.3 mm.

PITH.—In transverse section (fig. 18), the pith as preserved is 52 mm. wide and 32 mm. thick. It is therefore of unusually large size. There is no indication of septa such as characterize the large pith of most stems referred to as Cordaites. It is continuous throughout and has scattered within it numerous "sclerotic nests" which occur at irregular intervals. On the exposed surface of the pith at the proximal end, forty-two such nests are visible. In a transverse section about 2.5 cm. higher the number observed is considerably less. This may have no sig-

<sup>&</sup>lt;sup>1</sup> Received for publication April 24, 1939.

nificance since the number is variable in the serial sections cut from a portion of the stem. Although nests occur throughout the central region of the pith, they are absent in an outer zone of 3-5 mm. width adjacent to the secondary wood. Most sclerotic nests are approximately spherical in shape, and are composed of a group of polyhedral cells surrounding which the adjacent pith cells are elongated and so disposed as to form a conspicuous radial pattern as viewed in any sectional plane (fig. 10, 20). They vary from .1 mm. to 1 mm. in diameter (average .4 mm.). In most of them the central cells are not preserved except immediately adjacent to the pith and the central area may be more or less filled with brick-red granules which may represent decomposition products or resinous materials originally contained in the central mass. Commonly the central cavity is filled with calcite crystals.

In most sections the nests appear with greater frequency about 4 mm. from the secondary wood where they mark a fairly definite boundary between two pith zones (fig. 3)—a large central area where the cells as seen in transverse section are approximately equal in tangential and radial dimensions and a marginal zone where the pith cells are elongated radially. This elongation is particularly conspicuous between sclerotic nests and the secondary wood, between neighboring nests, and between marginal nests and nearby primary strands. The prominently elongated pith cells are relatively narrow and are darker than adjoining cells, which is apparently due to the presence of more organic matter. Such an arrangement and appearance suggest a primitive adaptation for conveying materials and indicate that the sclerotic nests may have been

AN — 1 — 2 — 3 — 4 — 5 — 6 — 7 — 8 — 9 — 10 — 11 ×

Fig. 2. Reconstruction of primary bundles from eleven sections cut in series from a block 2.2 cm. wide, as viewed from the stem interior. "xx" marks exit of superposed bundles. Reparatory strands on the anodic side connect with the thirty-fourth node above. Those on the kathodic side form a diagonal anastomosis.

glandular in nature. There are no secretory canals in the pith.

The average dimensions of the more central pith cells are as follows: height  $115 \mu$ , radial width  $150 \mu$ , and tangential width  $155 \mu$ . Radial elongation of cells in the marginal zone is accompanied

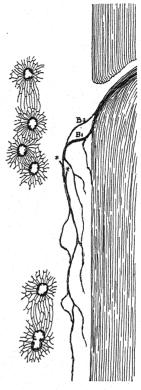


Fig. 3. Reconstruction of primary strands in relation to secondary wood and sclerotic nests, as seen in radial fractures. An outgoing leaf trace is shown at the upper right. B<sub>1</sub> and B<sub>2</sub> the two bundles which enter the trace. (\*) Reparatory and diagonal branches of the primary bundle. Small branches which appear to end blindly connect laterally with neighboring bundles.

by a corresponding reduction in other dimensions, which are as follows: height 97  $\mu$ , radial width 190  $\mu$ , and tangential width 83  $\mu$ .

Primary bundles.—As seen in transverse section, there are many small xylem strands embedded in the pith (fig. 11), all of which are submarginal in position. None are truly medullary such as have been described for Pitys (Scott, 1902) and Archaeopitys (Scott and Jeffrey, 1914). They are irregularly spaced, some are single, others occur in groups of three or four, and in some instances two or three distinct tracheid groups lie on the same radial plane. Many of the strands contain from two to four tracheid elements as seen in transverse section (fig. 13), and, where largest (near a node), they rarely have more than twenty cells (fig. 12). The larger bundles are tangentially elliptical.

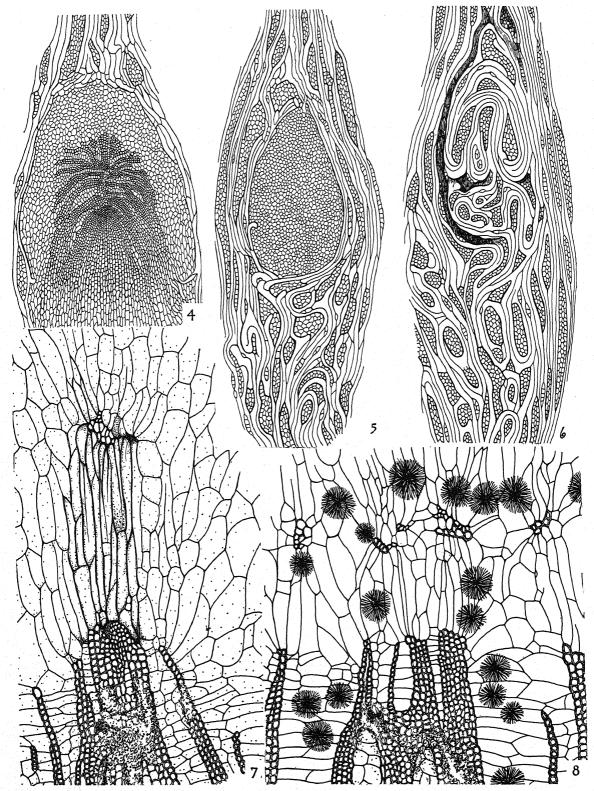


Fig. 4-8.—Fig. 4. Tangential section of secondary wood zone, showing emerging leaf trace cut transversely near its terminus. Secondary elements completely surround the primary wood. ×26.—Fig. 5. Tangential section of secondary wood cut about 1 mm. off the terminus of a leaf trace bundle. The general outline of the bundle persists in the mass of parenchyma. Tracheids on the abaxial side of the trace are much convoluted, and some of them are

A block 2.5 cm. long and 2.2 cm. wide was cut from the proximal end of the stem, and from it a series of eleven transverse sections were prepared to determine the course of primary bundles in the pith and their nodal relation to the paired leaf traces. Figure 2 is a diagram reconstructed from this series and shows approximately the course of primary bundles, except that it shows one only where two or more lie on the same radial plane. The figure represents the strands as seen from the center of the pith, so that the anodic direction or ascending spiral is toward the left. It is apparent that the strands diverged somewhat at the nodes, branched frequently, and connected with neighboring bundles. The two X symbols at the bottom of the figure mark the points of emergence of paired leaf traces which in section 11 are well out in the secondary wood, and the two above mark the position of the next pair of the same orthostichy. Although the two bundles in section 1 had not entered the secondary wood at that level, they were about to do so, as indicated by their size, shape, and the excessive elongation of pith cells between them and the secondary wood columns opposite. Included in the figure are three pairs of traces and four additional traces of other pairs.

Four bundle branches are formed at each node. Two are associated with each bundle. They are given off considerably below the node, one on the kathodic side and one on the anodic side of each bundle. The latter is a reparatory strand which continues apically, giving off one or two branches on the anodic side, and contributes to the correspending bundle which emerges at the next node of the same orthostichy (the thirty-fourth above). The branch which diverges on the kathodic side connects with the neighboring bundle by a diagonal anastomosis. There is this peculiarity in the arrangement due to the paired traces—the diagonal branch from the kathodic bundle of any pair contributes to the anodic member of the paired traces which emerge at the eighth node above, and that from the anodic bundle contributes to the kathodic member of the pair which pass out at the thirteenth node above. This diagonal connection therefore serves to join the anodic bundle of each pair with the kathodic member of the next pair of the same orthostichy and intercepts all branches within the distance of two superposed nodes. This arrangement appears to be a constant feature of the stem structure.

In the series of transverse sections, certain primary strands appear to be divided so that two or

more small xylem groups lie approximately on a radial plane. When traced upward or downward in the stem, these change their relative position and appear to connect again with the bundle or with the secondary wood. Radial sections confirm this evidence but are somewhat unsatisfactory because of the imperfect preservation and because the bundles follow a tortuous course.

Since the proximal end of the stem was fractured and subjected to weathering, and because of radial elongation of pith cells in the marginal zone, longitudinal-radial fractures could be made in this region of the stem. In this manner the nature of the slender xylem strands could be shown. They are seen to be branches from the primary bundles which connect with other branches, with the bundle at a higher level, or with the secondary wood. Figure 3 is a semi-diagrammatic restoration of this arrangement and was made from two fractures, one of which shows the bundle where it turns out from the pith to enter the wood zone as a leaf trace, and the other, from a lower level, includes the tangential divergence of reparatory and diagonal strands shown by the symbol (\*).

Since structural details are poorly preserved in the primary bundles, the identification of protoxylem elements is difficult, and must be determined largely by size rather than by wall sculpturing.

The bundles are largest near nodes where they contain about sixteen to twenty cells as seen in transverse section and appear in most instances to be endarch. In strands of four to eight elements the smaller cells may lie on the inner border, in a lateral position, or at the outer margin (fig. 8). The metaxylem elements are relatively small (average  $40 \mu$ ) and appear to be reticulate or transitional from reticulate to pitted. No distinctly pitted metaxylem can be observed.

A point of interest in the primary system pertains to the subsidiary branches or ramifications. Are they homologous with the true medullary strands of certain Pityeae (Scott, 1923) and of Archaeopitys (Scott and Jeffrey, 1914)? In the latter genus they are known to be branches given off by the primary bundles and have been described as developing centripetally and diverging upward. Whether or not they terminate in the pith or connect with other bundles at higher levels is not known. In this stem the small subsidiary branches tend to be radially aligned and in most instances lie between the primary bundle and secondary wood. They appear to be without protoxylem and never contain more than a few tracheids as seen in trans-

branched.  $\times 29$ .—Fig. 6. Tangential section of secondary wood cut about 2 mm. off the terminus of a leaf trace, showing the invasion of tracheids, convolutions, occasional branching of tracheids, and the short, rounded wood rays. The dark, curved column outlines one side of the gap which is here occluded with tracheids and distorted rays.  $\times 29$ .—Fig. 7. A transverse section cut at the point where the submarginal bundle is divided into radially aligned strands, one of which is in contact with secondary wood and the other is about to turn out. The two are connected by elongated pith cells. The outer strand appears to be endarch.  $\times 55$ .—Fig. 8. A transverse section cut below a node where the reparatory strand lies at the left (anodic position) and the diagonal strand at the right (kathodic position). The intervening bundle is composed of three tracheid groups. Note the variable position of protoxylem elements, the presence of spherulites, and dilation of rays.  $\times 55$ .

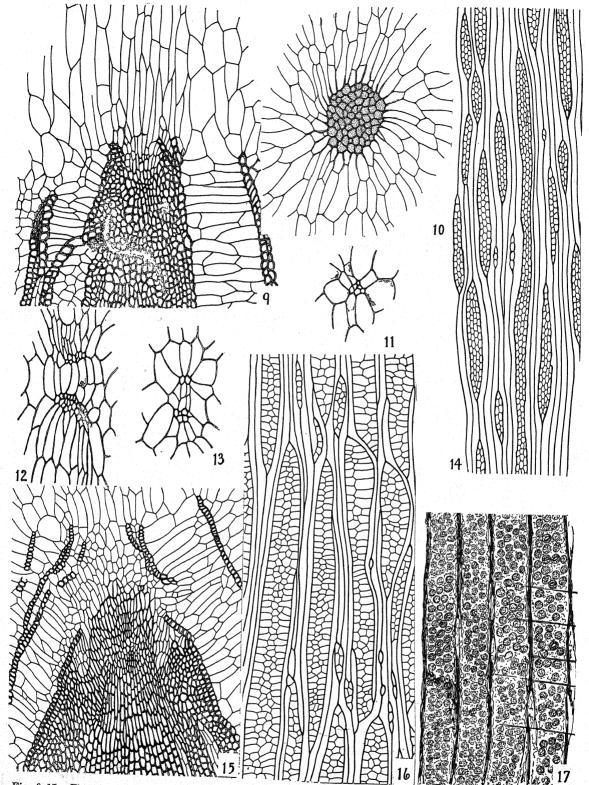


Fig. 9-17.—Fig. 9. Transverse section showing entrance of leaf trace into secondary wood. There are apparently two radially aligned protoxylem groups, cut at an angle and separated by larger elements. Secondary tracheids flank the primary xylem and close in behind the trace at higher levels. Pith is radially elongated opposite the trace. ×55.—Fig. 10. A radial section of pith and a sclerotic nest, showing the central mass of polyhedral cells and radially dis-

verse section. There is some resemblance to the medullary tracheids which in certain Calamopityeae have caused the central cylinder to be interpreted as being a modified protostele. In this instance, however, they are not isolated tracheid groups, since sections and fractures both show them to be continuous with the primary strands. So far as can be determined, all bundles lie within 2.5 mm. of the pith margin.

LEAF TRACES.—In figure 3 is shown the manner in which the primary strand divides near a node so the two bundles lie on the same radial plane one higher than the other. The lower turns out to the secondary wood sharply and is followed by the upper branch, which, so far as could be determined, holds this inner or adaxial position, and the two emerge through the secondary wood retaining this relative position. This manner of branching is observed in both sections and fractures and appears to be a regular nodal feature typical of both traces.

The vertical distance of disturbance in the secondary wood caused by the emission of a leaf trace bundle is about 6 mm. The trace therefore curves sharply and in the upper part of its course is almost horizontal in position. Because the distance between nodes is about 1.3 mm. and the traces pass through the secondary wood within a vertical distance of 6 mm., a transverse section of the stem will show five pairs of outgoing leaf traces, one or two of which will be obscure because they are in an early stage of emission. In figure 18 the traces are shown imperfectly, partly because of the transparent quality of the petrifaction. Their position has been marked in the figure as determined with a hand lens. The sequence as observed here, conforms with the arrangement shown in figure 1.

The four figures, 7, 8, 9, and 15, illustrate the changes which are typical of each bundle as it forms the anodic reparatory strand and diagonal kathodic bundle, then turns out to become a leaf trace. Figure 8 is a camera lucida drawing from a section cut about 5 mm. below a node at a point where the anodic and kathodic strands diverge and lie tangential to the main bundle. The smaller xylem elements are variously placed. In the anodic strand (shown at the left) the arrangement appears to be exarch, in the central strand endarch, and in the kathodic strand lateral in position. Secondary wood opposite the bundle is not organized into a column at this

level and apparently has no primary xylem at the inner margin. The general appearance of the spherulites is shown in this drawing and omitted from all others. Within the secondary wood zone a diagonal band of indistinct tissue is seen where the cells have broken down, a condition which is common locally in both primary and secondary wood.

Figure 7 was made from a cut at a higher level where the two branches have diverged widely and are not shown. The primary bundle is divided so the lower part is in contact with a column of secondary wood and is connected with the upper part (buried in the pith) by greatly elongated pith cells. The outer or largest branch appears to be endarch. Figure 9 shows a trace as it enters the wood column where the two groups of primary xylem elements are cut obliquely because they curve outward immediately upon entering the wood zone. Where a leaf trace enters the secondary wood, neighboring tracheids of the column are not at once carried out, but distally these lateral elements incline toward the primary xylem and farther out are caught up in the emerging bundle and carried out with it.

Figure 15 includes a trace buried in the secondary wood. Downward extensions of radially arranged secondary elements are shown closing the gap between the trace and pith. The primary elements being inclined at an angle are cut diagonally, which makes their arrangement obscure. There appears to be two groups of protoxylem surrounded by larger elements which are interpreted as being metaxylem. The arrangement is probably mesarch. The heavy border in the lower center marks the transition between secondary wood carried out on the abaxial side and the column beneath it which is not diverted.

The three figures, 4, 5, and 6, show the arrangement as seen in tangential sections cut near and beyond the point where the individual trace terminates in the secondary wood. Figure 4 is immediately proximal to the terminus. At this point there are two protoxylem groups vertically arranged and separated by metaxylem and some parenchyma. The bundle is mesarch. Rows of secondary elements completely surround the primary strand and have a loose arrangement due to the presence of wood rays. A prominent crescent of parenchyma overlies the bundle.

posed pith.  $\times$ 46.—Fig. 11. A small primary strand in a submarginal position, showing endarch arrangement.  $\times$ 50.—Fig. 12. Division of a primary bundle into inner and outer strands, immediately before turning out to the secondary wood to form a trace. Both bundles enter the leaf trace.  $\times$ 50.—Fig. 13. Division of a primary bundle to form a looped branch or ramification, composed of two elements which are apparently metaxylem.  $\times$ 50.—Fig. 14. A tangential section of secondary wood about 1 cm. from the pith. Rays are prevailingly high and wide. A few are uniseriate and low. Ray cells are typically higher than wide.  $\times$ 37.—Fig. 15. A transverse section of a leaf trace passing through the secondary wood zone. Note the narrow columns of secondary elements closing the gap behind the trace, the elongated pith cells, the protoxylem strands radially placed between the flanking columns of secondary wood, the presence of parenchyma in the trace, and the angle at which the trace is cut because of wide divergence from the vertical axis.  $\times$ 37.—Fig. 16. A tangential section of secondary wood within 1 mm. of the pith, where ray cells are dilated tangentially. The narrow secondary wood columns curve about the rays. Small uniseriate rays extend to the pith.  $\times$ 37.—Fig. 17. Radial section of secondary wood showing characteristic features of the pits and their arrangement.  $\times$ 220.

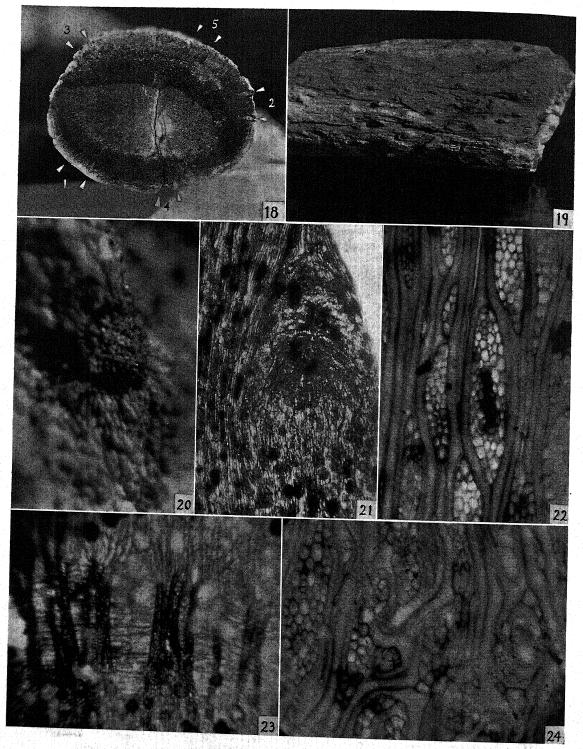


Fig. 18-24.—Fig. 18. A transverse section of the stem showing the large pith, narrow wood zone, and paired arrangement of traces. Traces which are obscure in the photograph are marked as determined with a hand lens.  $\times \frac{3}{4}$ .—Fig. 19. Side view of the stem showing the low protrusions due to convoluted tracheid masses which closed over the leaf traces.  $\times \frac{2}{3}$ .—Fig. 20. A sclerotic nest embedded in the pith, as seen on the surface of a fracture. In this instance the central mass of cells has not broken down.  $\times 50$ .—Fig. 21. An outgoing leaf trace cut near the terminus, showing the wide, short rays, abaxial to an outgoing leaf trace.  $\times 60$ .—Fig. 23. A transverse section of the pith margin and

Figure 5 was made from a section cut immediately off the terminus of a trace and shows a prominent mass of parenchyma which has the general outline of the bundle. Scattered tracheids occur in the mass which is probably callus tissue.

Figure 6 was made from a section cut about 1.5 mm. beyond a trace terminus and shows convoluted loops of tracheids which have closed in opposite the bundle terminus. The arrangement here resembles that found abaxial to the emerging trace in having numerous rays which are short, four or five cells in maximum width, and almost round. At two or three

places branched tracheids may be seen.

SECONDARY WOOD.—The zone of secondary wood has a maximum thickness of 13 mm. The preservation is such as to indicate that little of the secondary wood is lost. Apparently the stem when interrupted in growth was in its second growing season, since the leaf trace bundles are visible on all sides but are closed over and covered with about 2 mm. of wood in such a manner that the small protrusions

mark the points of emergence.

The wood is not dense like that of Cordaites and Eristophyton, but is characterized by broad wood rays (fig. 14, 16) such as are typical of Pitys (Scott, 1902) and Calamopitys (Scott and Jeffrey, 1914). Secondary tracheids are of medium size with an average tangential diameter of about 40  $\mu$  and a radial diameter of 44.5  $\mu$ . From two to six radial rows of tracheids lie between neighboring rays. The most frequent number is four. Where they meet the pith, rows of xylem elements are most frequently one or two cells wide and are not arranged in wedges or columns such as characterize Callixylon (Zalessky, 1909), except near or immediately below a node where a small column is formed partly by conjunction of neighboring xylem rows (fig. 23).

Pits are limited to the radial walls of secondary tracheids except immediately abaxial to outgoing traces where tangential pitting occurs sparingly. Pits are arranged in 1-3 vertical series, are uniformly distributed, and alternate in arrangement (fig. 17). Most of them are round and contiguous. Rarely in the larger elements they may be crowded and hexagonal. The pore is a narrow inclined slit, with the pit completely bordered. The characteristic features of pitting cannot be observed except locally in favorable areas. Throughout, most of the

stem pits are either obscure or invisible.

Wood rays vary from one to seven cells in width. The narrow and very wide rays are rare, while those of three or four cells in width are most common. Rays are high except near an outgoing leaf trace where they may be short or rounded (fig. 14, 16, 24). The maximum height observed is ninetytwo cells. Many are thirty to fifty cells high. No ray tracheids are visible, but occasional rays are composed of large cells or bordered by cells of unusual size such as have been described for Pycnoxylon (Cribbs, 1938), where they sometimes include tracheid elements. Where rays meet the pith, they are dilated, many of them in a striking manner (fig. 23). This is due to the tagential elongation of ray cells in that region. Farther out in the secondary wood ray cells are elongated radially and become muriform. Radial sections where they cut a dilated ray show the cells to be approximately square with the corners slightly rounded.

RELATIONSHIP.—This stem has a combination of structural features which make difficult its classification in relation to types previously described. This difficulty is increased because of the absence of cortex, leaf bases, and reproductive structures. The characters are a peculiar combination of those generally ascribed to genera of three groups-Cordai-

teae, Pityeae, and Calamopityeae.

The presence of a pith two inches or more in diameter, surrounded by a zone of secondary wood through which leaf trace bundles pass out to the cortex in pairs, diverging from the stem axis at a wide angle, are features characteristic of certain Cordaiteae (Scott, 1923). In contrast to these features many others of importance are indicative of relationship with the Pityeae. Among these are the following: wide wood rays, a large number of primary strands all of which are embedded in the pith near its margin, radial elongation of pith cells, wide dilation of rays near the pith, sparse pitting on tangential walls of tracheids abaxial to emerging traces, conspicuous distortion of tracheids in the neighborhood of outgoing traces, secondary tracheids surrounding the trace well out in the secondary wood, absence of rounded pits in the metaxylem of the primary bundle, and bundles of small size at the nodal position.

There are a few features which do not conform to the pityean plan. Of these the most important are: paired traces that originate from separate bundles, primary strands much reduced in the lower part of their course, an endarch tendency, reparatory strand lateral to the bundle, sclerotic nests in the pith, and subsidiary branches from the primary

bundles limited to the marginal zone.

Although sclerotic nests have not been reported for the Pityeae, this may not be significant, since they occur in different groups of paleozoic plants characterized by a large pith, including Lyginopteris (Kubart, 1914), Eristophyton (Scott, 1902),

and Mesopitys (Zalessky, 1911).

There is a resemblance to Eristophyton (Scott, 1902) in the presence of sclerotic nests, a continuous parenchymatous pith, and primary strands with metaxylem reduced in the lower part of their course. In other respects a comparison with this genus serves to emphasize the difference. Eristophyton differs in having primary strands in a marginal

secondary wood. Note the dilation of rays and ray cells, radial elongation of marginal pith, slender columns of secondary wood at the pith border, and the spherulites. ×20.—Fig. 24. Branched tracheids and rounded rays abaxial to an outgoing leaf trace. ×70.

position, bundles much enlarged at the nodes, metaxylem elements much larger than the secondary elements, metaxylem with many rows of rounded pits alternately grouped, leaf traces that originate and pass out through the wood singly, and rays that are

narrow or of the cordaitean type.

The double leaf trace which is typical of most cordaitean genera is also present in some of the Calamopityeae, including C. americana (Scott and Jeffrey, 1914), where it is formed by division of a single bundle at the node. The double trace arrangement of this stem resembles more closely that of Diichnia (Read, 1936), since each trace of the pair connects with a separate bundle at the inner edge of the secondary wood. It differs from Diichnia, in which two adjoining bundles give off leaf traces at each node, for in this stem the two which form traces at a node are regularly separated by two intervening bundles which may be either branched or simple. Diichnia has a 2/5 phyllotaxis, "mixed pith," mesarch primary bundles, and medium sized five angled pith, all of which are in contrast with corresponding structures of this stem.

There is a resemblance to both Calamopitys americana and Diichnia in having one of the traces of a pair slightly more apical or higher than the other, the difference varying from .5 mm. to 1 mm., in this stem, which is scarcely appreciable where the two traces have a tangential divergence of 1 cm. The wide high rays which have been compared with those of Pitys likewise resemble the rays of the more herbaceous Calamopitys species, except that in the latter there is less dilation near the pith.

There are occasional branched tracheids which lie abaxial to the outgoing trace and appear to be restricted to this region of excessive distortion. This feature has previously been reported for one species only—namely, Calamopitys eupunctata

(Thomas, 1935).

In regard to the geological horizon, this stem is somewhat later than the known Calamopitys species of semi-herbaceous habit, most of which have been reported from the Upper Devonian strata. It is approximately contemporaneous with Pitys and Eristophyton, reported from Scotland and Europe. Since it belongs to the lower part of the Mississippian, it preceded the great divergence of cordaitean forms of the Pennsylvanian. Whether or not it is too late to be in the line of descent between a Pteridosperm ancestor and the Cordaiteae is not in the writer's opinion a closed question, since it is not certain that all Dadoxylon types of secondary wood reported from the Devonian and Mississippian belong to the Cordaiteae. More evidence is needed regarding the primary structure, foliage, and reproductive parts of these early plants referred to Dadoxylon. It may well be true, that the Cordaiteae evolved from transitional types which flourished in the Mississippian.

The structural features of this plant are such that it may have been in the line which led eventually to the Bennettitales or Cycadales. Judging from the

meager evidence at hand, the stem was probably a stocky unbranched vertical axis, with densely clustered leaves of large size, the basal ones falling away as new ones developed above. Such is the general habit of the cycad with which a comparison reveals a certain degree of structural resemblance including a large pith, narrow wood zone, complex spiral, wide wood rays, and short internodes. The primary bundles which are greatly reduced in size may be considered as transitional between the more modern gymnosperms which lack them and the herbaceous type of Pteridosperm where they are prominently developed.

Because of the combination of structural characteristics this plant cannot at present be classified with certainty. Many important structural features indicate a place in the Pityeae, others of apparent equal importance indicate a place in the Pteridosperms, and whether it be included in one group or the other depends upon the relative weight given to the several primary structural features. In most respects the features which are not in agreement with the known Pityeae indicate affinity with the Calamopityeae rather than with the Cordaiteae. There is a possibility that further studies of the Reed Springs Flora will provide additional evidence through the discovery of closely allied plants.

Species and genus description.—Cauloxylon ambiguum, gen. et sp. n.—Pith very large (52 mm. wide), continuous, without ducts, with marginal zone of radially elongated cells, central zone with cells approximately isodiametric. Sclerotic nests abundant, limited to central pith zone, spherical, .4 mm. average diameter, surrounded by pith cells that radiate in all directions. Primary bundles distinctly separated, about 100 in number, all submarginal, much reduced at lower levels, about 16-20 cells at node, strong endarch tendency with protoxylem position variable in smaller bundles, anastomosis and branching common, subsidiary strands of 2-4 elements common and frequently radially grouped in relation to the primary bundle. Leaf traces paired; formed from separate bundles between which two others are intercalated; traces with two strands radially grouped; mesarch primary xylem; encircled by secondary elements distally; and about 200 -400  $\mu$  wide at the node. A reparatory strand and diagonal branch formed from each of the bundle pair, the former lies on the kathodic and the latter on the anodic side. Reparatory strands extend to 34th node above. The diagonal branches connect one with the 8th, and the other with the 13th node above. Phyllotaxis 20/34. Secondary xylem 13 mm. thick; tracheids 40 µ tangential  $\times$  44.5  $\mu$  radial diameter; rays wide and high, short and rounded adjacent to outgoing trace, widely dilated near the pith; certain rays entirely or in part of higher larger elements. Secondary tracheids adjacent to outgoing trace distorted and highly convoluted, occasionally branched or tangentially pitted. Pits of secondary wood uniformly distributed, alternate, 1-3-ranked, rounded and contiguous, rarely crowded and hexagonal. Pore an inclined slit, completely bordered. Metaxylem of trace with multiseriate pits, that of submarginal bundles about same size as secondary wood and transitional between reticulate and pitted.

#### SUMMARY

This stem is the third of a series to be described from the Reed Springs Formation of southwestern Missouri. It is a synthetic form combining characteristics of the Calamopityeae, Pityeae, and Cordaiteae. In most respects it resembles Pitys but differs sufficiently to justify separation from that genus. The points of difference for the most part indicate close affinity with the Calamopityeae. The stem fragment found was about 15 cm. long, 7.5 cm. wide, and 5.5 cm. thick. It has a phyllotaxis represented by the fraction 20/34. It is straight and without evidence of branches. It resembles Pitys in the following important respects: a very large pith, pith cells radially elongated, about 100 isolated primary bundles embedded in the pith near its margin, rays which are wide, high, and dilated near the pith, a limited amount of tangential pitting, metaxylem of small size and not fully pitted in the primary bundle, and outgoing traces completely surrounded by secondary tracheids distally.

The stem differs from Pitys and shows Calamopityean affinity in having many sclerotic nests in the pith, paired leaf traces which originate from separate bundles, leaf gaps with reparatory strand in an anodic position, reduction of metaxylem in the lower extension of bundle, and in the evidence of its having large leaves. Its definite placement in relation to the Pitveae or Calamopityeae is provisional, pending further studies of the Reed Springs

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# OSMOTIC AND PERMEABILITY RELATIONS IN THE NUTRITION OF FUNGUS PARASITES 1

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THE SPECIALIZED relationship which exists between such highly evolved parasites as the rust fungi and their hosts has been the subject of voluminous comment, but the physiological mechanism

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by which these fungi are able to obtain the necessary nutrients from the host cells, without causing any marked injury, has received little attention or explanation. The more destructive parasites which manifest the phenomenon of "action in advance" are considered to be able to satisfy their nutritional requirements from materials liberated from host cells which are killed by an enzyme or toxin secreted by, and diffusing in advance of, the encroaching mycelium; but the more highly specialized parasites such as the rust fungi employ no such drastic action.

Fundamental processes in the absorption of food materials by cells are those associated with osmotic pressure and permeability. Little direct investigation of these phenomena in connection with the feeding of parasitic fungi has been accomplished.

Steiner (1930) suggested that the osmotic value of urediniospores might offer a basis for differentiation of physiological forms of cereal rusts, but Hassebrauk (1932) later concluded that the method was inapplicable because of the many factors effective in modifying the osmotic value of spores.

Ronsdorf (1934) presents a value of 49.5 atmospheres for the osmotic pressure of urediniospores of *Puccinia triticina* and finds that urediniospore germ tubes of *P. simplex* have an osmotic pressure of 30.4 atmospheres while the value for cells of their host plant (barley) is only 9.5 atmospheres.

Rippel (1933) states that the rate of germination of spores of several fungi varies directly as the osmotic pressure of these spores. A Botrytis sp. parasitic upon Cladosporium fulvum had a much greater osmotic value than did the latter.

Hawkins and Harvey (1919) estimated the osmotic pressure of Pythium debaryanum to be 54 atmospheres. This pressure was found to be greater than the resistance to mechanical penetration possessed by cell walls of tubers of susceptible varieties but less than that of disease resistant varieties. Corner (1935) states that formation of haustoria by powdery mildews without piercing or killing the protoplasm of host cells indicates that the "turgor pressure in the penetration process is greater than that in the host cell." Pady (1935) makes a similar observation for rust fungi.

Graf-Marin (1934) states that spores of Erysiphe graminis will germinate at 90 per cent relative humidity and suggests that this indicates that these spores have a minimum suction pressure of 142 atmospheres. Yarwood (1936) later found that these spores would germinate in an atmosphere above C.P. sulphuric acid. Hence, it would seem that the minimum relative humidity for spore germination cannot always be considered as an index of osmotic pressure of spores.

Information bearing on the relative osmotic pressure of non-necrotic diseased tissue and of healthy tissue of plants infected by parasitic fungi is largely indecisive. In some instances conclusions have been drawn from observations of "plasmolysis" in fixed sections, which would seem to be of dubious accuracy. Tischler (1911) found that diseased tissue of pea plants attacked by Uromyces pisi had a higher osmotic pressure than healthy tissue. Allen (1923) states that when Mindum wheat is attacked by Puccinia graminis tritici, cells in the vicinity of the parasite are plasmolysed. The same writer (1926) reports that the hyphae of P. triticina induce an increased turgor in the adjacent cells of Little Club wheat. Dufrenoy (1928) states that cells of Arisaema triphyllum infected by Uromyces calladii became plasmolysed in an 8 per cent sucrose solution, while healthy cells of the same section remain turgid. Rice (1934), in studies on the same rust, finds no difference in degree of plasmolysis of diseased or healthy tissue at this or at higher concentrations. Hursh (1927) states that parasitism of wheat plants by Puccinia graminis effected no change in osmotic pressure as determined cryoscopically. Ruttle and Fraser (1927) found that oat plants invaded by *Puccinia coronata* show a heightened turgor among the cells immediately influenced by the rust's attack.

Attempts have been made to correlate varietal resistance to parasitic fungi with osmotic pressure of the host cells. Pantanelli (1921) and Johnson and Johnson (1934) in their studies on rusts of pea and wheat, respectively, reported a negative conclusion in each case. Shaw (1935) shows that resistance of apple trees to Bacillus amylovorus is correlated with the relative humidity in the intercellular spaces of host tissue as determined from the osmotic pressure of cells adjacent to the bacteria.

Measurements of permeability in relation to parasitism are few indeed. Fischer and Gäumann (1929), in a discussion of modified water relationships found in plants parasitized by rust fungi, suggest that alteration of permeability of the cell membrane, together with mechanical injury, bring about a disturbance of osmotic equilibrium which is responsible for the observed abnormalities. Link and Wilcox (1936) suggested that nutrients may be made more available to the fire blight bacillus (Bacillus amylovorus) by an increased permeability of host tissues caused by the parasitic activities of the organism. No measurements are presented. Thatcher (1935) demonstrated, by the plasmolytic method, that Uromyces fabae brought about an increase in permeability of the plasma-membrane of cells of its host (Pisum sativum) in the vicinity of the invading mycelium. Gretschushnikoff (1936) states that toxins elaborated by Puccinia coronifera cause increased permeability of leaf tissues of oat plants. The method of measurement was not stated in the abstract available.

The above observations are largely indirect and inconclusive. Accordingly, studies were made on the osmotic pressure and permeability relationships of various host-parasite associations with the hope of obtaining some information on the mechanism of nutrition, particularly of rusts.

MATERIALS AND METHODS.—In order to make comparison between organisms representing different degrees of specialization in their mode of parasitism, the fungi used in these studies were selected from two conventional groups—namely, (1) obligate parasites and (2) parasites which kill the cells of their hosts in advance of their mycelium. The species selected from these groups were, respectively, (1) Uromyces fabae (Pers.) de Bary, causing the rust of garden pea (Pisum sativum), and U. caryophyllinus (Schr.) Wint. on carnation (Dianthus caryophyllinus); (2) Sclerotinia sclerotiorum (Lib.) Mass. and Botrytis cinerea Pers. on celery (Apium graveolens). By means of plasmolytic methods the osmotic pressure of the respective hosts and parasites was compared, and the influence of the various fungi on the permeability and osmotic pressure of the host cells was determined by comparing these properties at different distances

from invading hyphae. The precise technique had to be modified according to the materials used, as reported below.

Osmotic pressure determination.—For ease of observation, all preparations were vitally stained with neutral red, 200 p.p.m. in tap water. Calcium chloride was always used as plasmolyte, the solutions

being made up from anhydrous CaCl<sub>2</sub>.

The osmotic pressures of urediniospore germ tubes were determined by the method of Ronsdorf (1934) who considered as isotonic with the germ tubes of Puccinia triticina the strongest solution in which the spores would just germinate. Urediniospores of Uromyces fabae were floated on calcium chloride solutions contained in Van Tieghem cells which were kept in moist chambers. Germination was estimated after 18 hours. The solution considered isotonic with the spores was the one in which initial germ tube protuberances were visible to the extent of 50 per cent of the proportion of germ tubes which would develop in tap water.

The osmotic pressures of haustoria of Uromyces fabae and of U. caryophyllinus, and of hyphae of Botrytis cinerea and Sclerotinia sclerotiorum, were determined by the plasmolytic method. Incipient plasmolysis of haustoria could be observed directly in sections of infected tissue placed in the appropriate concentration of calcium chloride. Some difficulty arose in ascertaining the osmotic value of hyphae of Botrytis and Sclerotinia because of the great inequality in proportion of vacuolar space in different hyphae and because partial collapse of the hyphal wall was sometimes brought about by hypertonic solutions.

The isotonic value of very elongate hyphal cells with relatively large vacuoles - which generally showed a "convex" type of plasmolysis-was arrived at by first measuring the lengths of a number of successive vacuoles in a particular filament, while mounted in water. These same vacuoles were watched carefully during irrigation with progressively stronger solutions of salt until shrinkage (plasmolysis) of the vacuoles began. The hyphae were then more strongly plasmolysed and after equilibrium had been attained were further irrigated with progressively weaker solutions until the original sum of vacuole lengths was reached. The average between the respective mean concentrations at which shrinkage first appeared and at which the original length was regained was considered as isotonic with the fungus filament.

Those hyphae which were rich in protoplasm and in which the many minute vacuoles could not be measured, commonly plasmolysed in a "concave" fashion—i.e., the protoplasm receded from the walls at irregular intervals. With such hyphae, the isotonic solution was regarded as the one in which all these concave identations had just disappeared during deplasmolysis.

Camera-lucida drawings of hyphae plasmolysed in concave and convex manner are shown in figures 1B and 2, respectively.

Osmotic pressure of the host cells.—The osmotic pressure of host cells was determined in all cases by the incipient plasmolysis test. Thin free-hand sections were immersed in calcium chloride solutions of different concentrations and the number of cells showing some degree of plasmolysis and the number not plasmolysed, in fields totaling at least 100 cells, were determined after 30 minutes. The percentage number of cells showing plasmolysis in each concentration was then plotted against concentration, and the concentration at which 50 per cent of the cells were plasmolysed (the isotonic value) was determined from this graph.

In addition to the above method, the osmotic pressure of infected and non-infected tissues from rusted pea plants was also obtained by Höfler's (1931) "plasmometric" method. For cells of cylindrical shape, a closely approximating value for the degree of plasmolysis brought about by a hypertonic solution is indicated by the expression [l-d/3]/h, where l = length of plasmolysed protoplast, d = width of plasmolysed protoplast, and h = original length of protoplast before plasmolysis. The product of this quotient and the concentration of the plasmolysing solutions gives the isotonic value of the cell sap before plasmolysis (see Scarth and Lloyd, 1931).

Permeability determinations.—Comparative values of permeability of host cells in tissues invaded by fungal hyphae and of cells well removed from the infected zone were obtained with respect to water, urea, thio-urea, and dextrose. These substances form a series of increasing molecular size and decreasing rate of penetration and are therefore useful in estimating the pore size of the plasmamembrane. They are also substances of a type similar to those which may serve as food for the fungus and which, in whole or part, occur in the host cells. The presence of urea is problematical. The existence of urease in cells is regarded as indicating the speedy breakdown of any urea which may be formed, and, indeed, urea could hardly be expected to accumulate because of its ready passage through the protoplasm. However, changes in urea permeability are no doubt indicative of similar changes toward other highly diffusible nitrogenous compounds. The principal object of the investigation was to find out if any degree of permeability is developed toward nutrient solutes which the host cells contain in quantity and to which healthy cells are "passively" semi-permeable. Dextrose was selected as representative of this class of substance.

The cells compared were from the same organ and the same zone of tissue. The relative permeability of similar cells in the respective regions is all that is required for the present investigation, but the absolute or "protoplasmic" permeability—that is, the permeability per unit area of protoplast—has also been calculated. This latter value is particularly useful since it permits universal comparison of permeabilities of cells from different tissues and

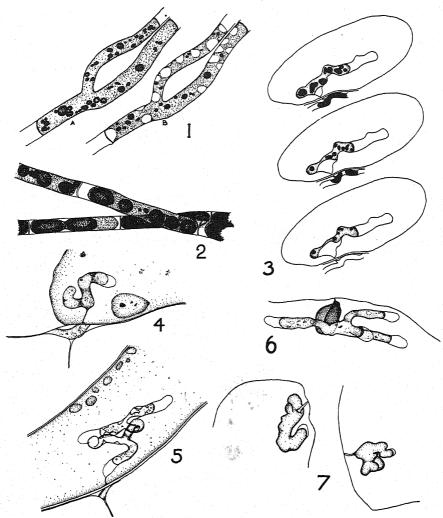


Fig. 1-7. (Camera lucida drawings.)—Fig. I. A. Hypha of Botrytis before plasmolysis; vacuoles stained with neutral-red. B. After plasmolysis; concave invaginations of the cytoplasm apparent.—Fig. 2. Hyphae of Sclerotinia plasmolysed in normal convex manner. Vacuoles stained with neutral-red.—Fig. 3. Progressive plasmolysis of the same haustorium as the concentration of plasmolyte was increased, presenting evidence that the phenomenon observed was true plasmolysis. Vacuoles in black. Host cytoplasm not figured.—Fig. 4. Convex plasmolysis of a simple haustorium.—Fig. 5. Concave plasmolysis in a haustorium. Not sufficient of the host cell shown to indicate its plasmolysis.—Fig. 6. Convex plasmolysis in a digitate haustorium.—Fig. 7. Haustoria as adjudged in the condition of incipient plasmolysis.

species, irrespective of the dimensions of the cells considered.

Calculations of passive permeability as measured by the plasmolytic method are based on the change in the volume of a protoplast occurring in a known time due to diffusion between the vacuolar solution and the external solution. This is most conveniently arrived at by measuring the increase in volume during deplasmolysis of a protoplast which has previously been plasmolysed.

Where the test substance is a solute, any increase in volume which occurs while the cell is immersed in a hypertonic solution is due to the fact that penetration of the solute increases the amount of osmotically active substance within the cell so that water is drawn into the cell. The volume change depends on the amount of solute entering.

If water is the test substance, volume change is measured while water diffuses from a hypotonic solution of known strength. The volume change may be obtained either by linear measurement with the aid of an ocular micrometer or by estimation after the cell has been plasmolysed initially to a known degree.

The time taken for deplasmolysis of cells of similar dimensions after they have been plasmolysed to the same degree gives a measure of relative permeability. Absolute permeability may be calculated from consideration of volume change within a given time under conditions of known osmotic pressure

difference. As is indicated by Scarth (1939), the rate of volume change at any moment, dv/dt, is expressed by the following general formula: dv/dt = p.c.s., where p = the coefficient of permeability of the protoplasm, c = the difference between the effective concentrations inside and outside of the protoplast, s = surface area of the protoplast.

Scarth shows that with proper precautions p in the above formula can be kept practically constant throughout deplasmolysis and that the variables c and s can often be expressed in terms of v—a condition which is necessary for the solution of the equation.

An obstacle inherent in calculation of permeability is the difficulty of estimating s and v, which is only possible when the shapes of the cells tend to a definite geometrical form.

Table 1. Rate of deplasmolysis of exposed cells and of cells partly "blanketed" by adjacent cells. Petiole tissue.

Time for deplasmolysis						
Per	meabili	ty to wa	Pe	Permeability to urea		
Exposed cells		Blanketed cells			Exposed cells	
Mins.	Secs.	Mins.	Secs.		Mins.	Mins.
5	0	7	40		49	<b>49</b>
3	10	7	40		48	50
6	40	7	40		491/2	50
7	10	7	0.0		48	48
7	40	7	00		46	48
7	40	6	20		52	51
7	40	6	20		$50\frac{1}{2}$	50
6	40	5	00		51	55
6	50	5	30		47	471/2
6	40	5	30		51	511/4
3	10	5	00			
4	30	7	00			
5	00	3	45			
5	20	4	20			
5	30	6	40			
3	15	7	05			
7	15	3	<b>T</b> 5			
3	30	3	45			
3	30	5	00			
7	15	5	30			
5	40	5	48	(Average)	491/4	50

Scarth expands the above equation and derives formulae comprised only of easily measurable factors, applicable to cells approaching cylindrical, cylindro-prismatic, and spherical form. By ingenious substitutions, Scarth has reduced these formulae to simple expressions which in some instances curtail the measurements necessary and which give results well within the limits of experimental error unless applied to cells having a low ratio of length to breadth. The formulae can be made rigorous by reference to a table of corrections presented in Scarth's paper (1939).

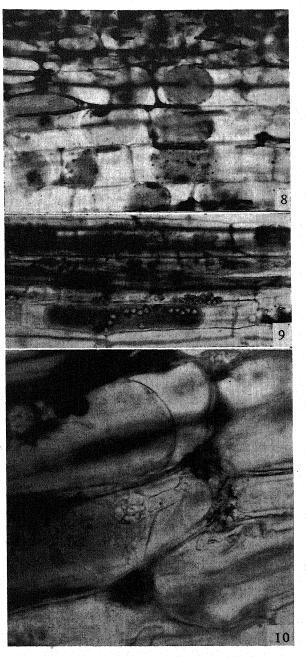


Fig. 8-10.—Fig. 8. Cells of the type commonly used for determination of permeability by the plasmolytic method.
—Fig. 9. The type of cell chosen for determination of permeability by the plasmometric method. Note the high proportion of length to diameter.—Fig. 10. Plasmolysis of an infected host cell without plasmolysis of the haustorium, indicating the superior osmotic value of the haustorium.

The sections were cut thin, so that, for the most part, they were only one cell thick. As a consequence, no blanketing effect on diffusion rate was manifest even in cells situated in the middle of a section. Table 1 shows that, on the average, the latter deplasmolysed at exactly the same rate as cells at the edge. This means that available surface (s in the primary equation) is the whole surface of each cell. The table also shows the degree of individual variation among the cells. This is considerable when cells are rapidly deplasmolysed in hypotonic solutions, as used in measuring water permeability, but is slight in gradual deplasmolysis as in hypotonic urea solution. Probably the variability shown in the former represents not so much normal variation of permeability as abnormal variation caused by rapid stretching of the plasma membrane, since always a certain number of the cells showed injury in deplasmolysis. To equalize this mechanical effect as much as possible, the sections compared were brought to the same degree of plasmolysis, as is described later.

In shape all the cells studied were more or less cylindrical. Those selected for the most important tests were about five times as long as broad. The bulk of the tissue in all preparations, however, was made up of cells only from three to four times as long as broad, and it was with such cells as well as with the narrower ones that most of the tests were made. The results presented have been calculated from Scarth's simplified formulae using the correction indicated in Scarth's table.

Details of the experimental procedure are as follows:

Water permeability.—The osmotic value (O) of the cells having been determined, the cells were plasmolysed for 20-30 minutes in CaCl<sub>2</sub> of conc. 2 O. They were then transferred to a solution of conc.  $\frac{1}{2}O$ , and the time (t) required for completion of deplasmolysis of 50 per cent of the cells was noted. The average diameter of the cells (d) was also determined. P (absolute permeability) = d/5 t O (Scarth, 1939).

Urea permeability.—In using the plasmolytic method for measuring solute permeability, the osmotic value of the cells was first determined, as described before. The sections were then transferred to a solution of penetrant of concentration 2 O. (As

plasmolysis was smooth and convex, this procedure is free from the objections that apply when there is much adhesion to the wall.) The time for completion of deplasmolysis was noted and the average diameter (d) of the cells determined. In this case absolute permeability to solute (Ps) is given by the formula Ps = d/4t.

Permeability to dextrose—plasmometric method. In a solution such as that of dextrose which normally penetrates cells very slowly or not at all. complete deplasmolysis, if ever it occurs, is likely to be preceded by changes in the osmotic pressure and permeability of the cells. The plasmometric method gives results in a small fraction of the time required by the plasmolytic. In using this method, the cells were plasmolysed in a dextrose solution, and after equilibrium was reached the length of the protoplast (lmin.) was measured. After a suitable time the length of the same protoplast was again measured (1). In experiments in which it was desired to estimate the average permeability of a number of cells which could not always be recognized individually with certainty, the average length l was corrected in proportion to the ratio of L (average) in the second set to L in the first set of measurements. L being the length of the entire cell.

In this case  $Ps = [(l-l_{\min}) d]/4 t l_{\min}$ 

RESULTS.—Osmotic pressure.—Host v. parasite.

—The osmotic pressure of each of the parasites investigated was found to be considerably greater than that of its host, as seen in table 2. MacDougall (1911) and Harris (1916) have stated that such a relationship is general between angiospermous parasites and their hosts.

The value obtained for germinating urediniospores of *U. fabae* (vide table 2) is in close agreement with the value presented by Ronsdorf (1934) for *Puccinia triticina*: *U. fabae* 43.5-45 atmospheres, *P. triticina* 43.9 atmospheres. The method of estimation, however, is indirect, and it is not unlikely that these values, which are high for plant cells, may be accounted for partly by an adaptation of the spores to the abnormally high osmotic pres-

Table 2. Osmotic pressure of parasite and host.

Parasite		Host			
Fungus	Average O. P. in atmospheres	Host plant (healthy tissue)	Average O. P. in atmospheres		
Uromyces fabae Germ tubes Haustoria		Pisum sativum Leaf Petiole Stem	10.10		
U. caryophyllinus Haustoria	18.6	Dianthus Leaf base	11.2		
Botrytis cinerea Hyphae	29.8	Apium graveolens Petiole	8.3		
Sclerotinia Hyphae	23.5	Apium graveolens Petiole	13.4 (9.4–17.4)		

sure of their environment during the relatively long period of immersion in the test solutions (cf. Walter, 1934).

The average osmotic pressure of haustoria of Uromyces fabae and U. caryophyllinus was found to be 21.9 and 18.6 atmospheres, respectively. The relatively high osmotic pressure of haustoria of U. fabae, as compared with that of host cells, is subtantiated by figure 10, which shows a host-cell strongly plasmolysed without any plasmolysis of the haustorium being apparent. Evidence that the shrinkage of the protoplast observed in haustoria after immersion in hypertonic solutions was true plasmolysis and not an artifact is afforded by the camera-lucida drawings in figure 3. These drawings show the same haustorium, with vacuoles stained by neutral-red, at increasingly hypertonic concentrations of calcium chloride. The progressive contraction of the vacuoles is readily apparent. Cameralucida drawings of haustoria adjudged to be in the condition of incipient plasmolysis are shown in figure 7.

Infected vs. non-infected tissue.—Analysis of more than 300 measurements of cells from tissues interspersed with hyphae of *U. fabae* and from non-infected tissues of the same organ indicates a significant decrease of more than two atmospheres in the osmotic pressure of infected cells as compared

Table 3. Average osmotic pressures of infected and noninfected tissues of Pisum sativum (compiled from more than 300 measurements).

	Average O atmosph	D:W	
Tissue examined	Non-infected	Infected	Difference in O. P.
Petiole 1	10.1	7.78	2.32
Petiole 2	9.18	7.00	2.18
Petiole 3	11.03	8.25	2.78
Stem	10.26	8.77	1.49
Leaf	9.15	6.74	2.76

with non-infected cells. Summaries of these measurements are presented in table 3.

Permeability.—Permeability to water.—Marked increase in water permeability in tissues infected with U. fabae is indicated in table 4. In petioles, the rate of penetration of water into cells of infected tissues was more than five times as fast as in healthy regions of the same organ. In leaf tissue (palisade cells) almost a threefold increase was noted. Sudden disruption of the cytoplasm in cells of infected leaf tissues occurred frequently during deplasmolysis. This probably indicates that the reported measurements are those of cells least modified by the presence of the rust hyphae.

In an attempt to determine whether or not this increase in permeability was brought about by a secretion from the fungus, sections of non-infected pea tissue were immersed in extracts from equal weights of leaf tissue, (1) from regions densely packed with hyphae of *U. fabae* and (2) from non-infected areas of the same leaves. Permeability measurements of these immersed tissues made at the conclusion of the period of immersion indicate that the permeability of the cells to water was increased much more by the extract from infected tissue than by the extract from healthy tissue (see table 5).

Examination of celery tissues attacked by Sclerotinia and Botrytis reveals the fact that each fungus causes an increase in permeability, a gradient in change being noticed from tissue in the immediate vicinity of the fungus to healthy tissue some distance away from the infection center (table 6). This change precedes death of the cells.

Permeability to urea and thio-urea.—Substantial increase in permeability of cells in infected tissues to urea and thio-urea as a result of parasitism by U. fabae and U. caryophyllinus is indicated in table 4. A graphical expression of the increase in permeability to urea by carnation leaf-base cells infected with U. caryophyllinus is given in figure 11. The curves were obtained by expressing rate of deplasmolysis in a strongly hypertonic solution in terms of the percentage of cells having completed

Table 4. Permeability\* of host tissues to water, urea, and thio-urea in relation to rust infection. Plasmolytic method.

	Permea-		Non-infected tissue Infected ti			
Fungus	bility test material	Host tissue	Deplas. time	Permea- bility	Deplas. time	Permea- bility
Uromyces fabae	Water	Pea				
		Leaf	$4\frac{1}{2}$ mins.	97	134 mins.	250
		Petiole	11¼ mins.	58	2 mins.	325
	Thio-urea	Pea	Hrs. Mins.		Hrs. Mins.	
		Petiole 1	9 46	0.0010	2 14	0.0044
		Petiole 2	15 9	0.0011	6 33	0.0026
		Petiole 3	11 17	0.0013	2 41	0.0053
U. caryophyllinus	Urea	Carnation				
J. J		Leaf base	17 0	0.0014	2 30	0.0098

<sup>\*</sup> Permeability to water expressed in  $\mu$  per hour per atmosphere. Permeability to solutes expressed in millimols per cm<sup>2</sup> per hour per mol concentration difference.

Table 5. Pea rust. Effect of juice expressed from infected and non-infected tissues on water permeability of healthy tissue.

		Deplasmolysis time			
Tissue	Period of immersion	Non-infected extract	Infected extract	Check	
Stem Leaf	2 hours 1 hour 1½ hours	9 mins. $3\frac{1}{2}$ mins.	5 mins. 2 mins. $1\frac{1}{2}$ mins.	10 mins. 3 mins.	

Table 6. Water permeability of celery tissue as affected by Botrytis cinerea and Sclerotinia sclerotiorum.

	Average deplasmolysis time			
Tissue	fected	ue in- l with rytis	Tissu fected Sclere	with
	Mins	. Secs.	Mins.	Secs.
1/4 inch below necrotic zone	1	34	1	58
I inch below necrotic zone	2	29	2	19
2 inches below necrotic zone			2	28
4 inches below necrotic zone	5	23	6	30
Uninfected petiole		7 mins. 5	7 secs.	

deplasmolysis at progressive time intervals. Each point on the curve represents observations of at least 100 cells.

Permeability to dextrose.—Values given in table 7 for permeability to dextrose indicate that after subjection to the parasitic activity of a rust fungus the

RATE OF DEPLASMOLYSIS: CARNATION TISSUE IN ISM URIA

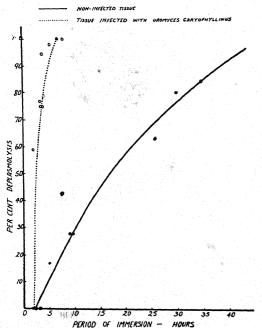


Fig. 11. Greater permeability to urea of rusted as compared with healthy carnation tissue as shown by relative rates of deplasmolysis in 1.5 M urea solution.

Table 7. Permeability to dextrose of cells infected by Uromyces fabae. Plasmometric method.

		Length		er and the second
		of prote	)-	
	Minimum	plast		
Deplasmolysis	length of	after	Diamete	r Permeability
time	protoplast	time $t$	of cell	millimols/
(t)	(l)	$(l_2)$	(d)	cm2/hour/mol
121/4 hrs.	329	367	47	0.00011
6 "	167	177	38	0.00010
6 "	136	144	30	0.00007
121/2 "	141	169	. 56	0.00022
251/2 "	141	188	56	0.00018
18 "	207	263	47	0.00017
5ª "	134	159	66	0.00061
5ª "	127	141	38	0.00010

<sup>a</sup> Values from average of 13 cells of which individual protoplasts were not recognized for measurement of  $l_2$ . Length  $l_2$  of the protoplasts after time t is corrected in the ratio of the average total cell length L of the second group of measurements after time t to the average total length L of the first group.

host cell membrane is modified in such a way as to permit dextrose to diffuse through it in measurable quantity, the permeability rate in this instance averaging about 0.00020 millimols pere square centimeter per hour per mol concentration difference.

Discussion.—Considering first the above measurements relative to rusts, the facts brought to light by the study of osmotic pressure and permeability suggest an explanation of how the rust fungi *Uromyces fabae* and *U. caryophyllinus* are able to obtain nutrients from the cells of their hosts without killing these cells.

As regards water absorption, the determining factor is of course suction pressure. Although this is not identical with osmotic pressure, yet in the relationship between parasite and host cell the maximum suction pressure which each of the two competitive organisms could exert would be equivalent to its respective osmotic pressure. Also, with equal turgor pressure, suction pressure varies directly with osmotic pressure. Hence, with its higher osmotic pressure, the fungus is able to remove water from neighboring parenchyma cells and can even maintain a certain degree of turgor in contact with non-turgid host cells or with their free sap.

As regards the absorption of foodstuff by the parasite from the cells of its host, the passive permeability of the latter would seem to be all important. We have seen that in all cases the fungus brings about an increase in permeability of the host cells, which is probably due to some sort of secretion, as indicated by the increase in permeability brought about by juice expressed from infected tissue. This increased permeability as measured by deplasmolysis extends even to dextrose so that probably some of the vacuolar solutes which are normally prevented from escaping become available to the fungus. In a non-turgid cell those components of the vacuolar solution to which the membrane has become permeable would diffuse out into the free water in the cell walls till its concentration there reached that in the sap. The amount would be relatively small. In turgid cells, however, in consequence of the reduction in osmotic pressure, water also will be forced out by wall tension. This mass outward flow of water and vacuolar solutes will also be checked when the reduced wall tension again balances the effective osmotic pressure so that cells do not lose turgor completely and remain alive.

The hypothesis that even an obligate parasite causes certain substances to leach from the host cells in its vicinity is supported by the fact that there is a reduced osmotic value in the tissues of Pisum which adjoin the rust hyphae. A reduction in osmotic value as tested by plasmolytic methods signifies a reduction of osmotically active solutes and is independent of changes in water content.

It is not intended to be inferred from the above discussion that change in permeability is the only physiological modification of the host cell essential for rust nutrition. Neither is it suggested that dextrose is the carbon source utilized by the rust fungi. It is certain, however, that whatever the compound used by the rust fungi, it must be one to which the host cell loses its semi-permeability, since the protoplasts of hosts and fungus are always distinctly separate. Dextrose is considered here only because it is representative of the type of diffusible carbon compound known to occur within cells.

The above hypothesis also sheds some light on a number of questions relative to the rusts for which no satisfactory explanations have been offered.

Several workers, including Smith (1900), Rice (1927), and Pady (1935), have shown that certain haustoria-producing parasites can develop an extensive amount of mycelium before forming haustoria, the development being far more than is possible by use of only reserve food contained in the spore. Arthur (1929) points out, also, that certain systemic rusts, in keeping pace with the development of the host, mingle with the meristem tissue, but do not form haustoria until cells at the growing point have become vacuolate. Such instances indicate that these fungi may obtain adequate nutrients to support rapid growth without use of specialized absorbing organs.

Each author asks by what means do these fungi procure their nourishment. The above explanation presents an answer. It may be extended to haustorial absorption if the common view is correct that the typical haustorium causes an invagination of the protoplasmic layers of the host cell and is not directly in contact with the vacuolar sap. Its function would then be to offer increased absorbing surface and to occupy a position favorable for immediate up-take of any material which "leaches" from the vacuole as a result of the membrane-modifying activities of the fungus.

Another phenomenon which may be interpreted in the light of the above is the commonly observed fact that a rust fungus develops best when environmental conditions favor maintenance of a state of high turgor in the tissues of its host. As already described, the rate of flow of vacuolar solution from infected protoplasts would be in direct proportion

to the turgor of the host cell.

Referring now to the destructive parasites Botrytis and Sclerotinia, the results presented in tables 2 and 6 indicate in each case that (1) the osmotic pressure of the fungus is greater than that of its host and that (2) permeability of the host plasmamembranes is increased. Thus, in the tissues as yet unaffected by their characteristic pectinase digestion, these organisms show the same type of influence as do the obligate parasites. The ability of these fungi to satisfy their water and food requirements during the initial period before pectic hydrolysis has reached completion, may perhaps be explained by the same hypothesis, since, in the case of celery tissue, at least, a decided increase in permeability is noted some distance away from killed cells. In addition, these permeability changes may well be influential in contributing to the total food supply after actual invasion of the tissues.

The difference in the relative pathogenicity of the two groups may be partly due to an adaptation whereby the rust fungus, in evolving a more specialized form of parasitism, has reduced the intensity of the factor through which the permeability of the host cells is increased and which, in the case of the "grosser" parasites, brings about an ultimately fatal modification. The view that a nonlethal action is to the advantage of the rust organism in maintaining continuance of its food sup-

ply is familiar.

#### SUMMARY

The osmotic pressure and permeability relationships between a number of parasitic fungi and their hosts were studied by plasmolytic methods.

The fungi used were Uromyces fabae on Pisum sativum, U. caryophyllinus on Dianthus caryophyllus, Botrytis cinerea and Sclerotinia sclerotiorum isolated from decaying celery petioles (Apium graveolens). Osmotic values were obtained for urediniospore germ tubes and haustoria of the rusts, for hyphae of Botrytis and Sclerotinia, and for diseased and healthy tissues of the various host plants. In all cases the osmotic pressure of the fungus was greater than that of its host.

Permeability measurements of diseased and healthy tissues in rusted plants indicate that the rust increases the permeability of the plasma-membrane of the host cells. Values for absolute permeability to water, urea, and dextrose were calculated by the use of the simplified formulae devised by Scarth (1939). This increase appears to be due to some secretion from the rust fungus as indicated by the increase in permeability brought about when healthy tissues are immersed in a juice-extract from tissues interspersed with rust hyphae.

An increase in permeability is also apparent in celery tissue some distance from the region of cells

killed by Botrytis or Sclerotinia.

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Based on the above measurements, a hypothesis is elaborated to explain the mechanism for transfer of food and water from the host cells to those of the parasite as manifest in the above rusts. This hypothesis permits an explanation of how host cells are able to remain alive even though being continually taxed by the parasite, and it throws light on other hitherto unexplained phenomena.

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# TWO NEW GENERA OF OPERCULATE CHYTRIDS 1

### Helen Berdan

In June, 1938, several saprophytic chytrids were noticed in a small piece of dead grass found in a collection of algae made in a pasture near Chapel Hill, North Carolina. Subsequent examination of fresh, boiled grass added to this showed the presence of seven chytrid species. Two of these were identified as Nowakowskiella elegans (Now.) Schröter and Cladochytrium replicatum Karling. Another was a small "dentigerate" form, and a fourth appeared to be a large, hyaline Cladochytrium species with exceptionally long exit tubes. The remaining three seemed sufficiently unusual in character to merit isolation from the confusing entanglement of the combined seven rhizoidal systems present in the grass tissue. To those familiar with the chytrids, such a separation may present no difficulties. To others, who, like the writer, are initiates in this field, the purely practical procedure may be both interesting and instructive.

The conditions of culture and study have been kept as nearly sterile as possible. All instruments, slides and cover glasses are flamed, all Petri dishes dry sterilized, all cultures maintained in sterile charcoal water made as described by Shanor (1937), on boiled substrata, and all washings done with distilled water. This and the purification methods described below were the procedures in the Chapel Hill laboratory where these cultures were begun (Couch, 1939). Two labor-saving innovations have been added. The laboratory at the University of Western Ontario is equipped with an air line from a compressor. Rubber tubing attached to the spigot conducts the air through a solution of HgCl2, then into the wash bottle. The continuous stream of water washes off rotifers, etc., with greater despatch and thoroughness than the ordinary wash bottle. We find that, with one careful washing, cultures may be kept in a cool room for several weeks in clean condition. Secondly, the leaves are boiled, then heated gently in 95 per cent alcohol in a water bath to remove the chlorophyll. These are stored on ice in stoppered flasks containing clean alcohol, which is then boiled out directly before using. Observations are made more easily in leaves treated in this way and chances of contamination reduced to a minimum.

The original culture was kept by the addition of new pieces of sterile leaves, a wise precaution, since during attempts at purification the chytrid may be lost. "Gross cultures" were obtained by washing pieces of infected leaves removed from the original dish, transferring them to sterile Petri dishes con-

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taining sterile charcoal water, and adding fresh sterile pieces of leaf.

From gross cultures, a culture in which one chytrid predominates (the "accumulation culture") may be derived. When one has sufficient skill to isolate a single spore or sporangium from the gross culture, this intermediate stage may be discarded. However, while the experimentation necessary to achieve an accumulation culture is sometimes tedious, the process is effective in familiarizing one with the growth habits and morphology of each chytrid in the gross culture. It affords opportunity for a comparative study of these on a range of substrata and under a variety of conditions. It also increases the chances of observing heterothallism, which may not be noticed if a single strain is established too soon.

In the present case it was decided to discard the "dentigerate" form, Nowakowskiella elegans (Now.) Schröter, and Cladochytrium replicatum Karling deliberately and to acquire accumulation cultures of the other four if possible. The general

lines of procedure are described below.

1. Portions of leaves containing the "dentigerate" form were cut off and discarded. Since it was present in small quantities only, it was soon removed from the gross cultures and made no reappearance.

2. Nowakowskiella grew very quickly in the hot climate and completed its cycle more rapidly than the others in the gross cultures. When washings were made less frequently, it tended to die out of the cultures, and after a few weeks' time it was completely eliminated. (We have found that an accumulation of this chytrid may be obtained by pulling off rhizomycelium which is growing out into the water from the substratum and placing it on fresh substratum (Haskins, 1939).

3. The large Cladochytrium species with very long exit tubes grew very slowly. It seemed to thrive only in leaves of a certain grass found in Chapel Hill. It succumbed to the exigencies of travel from North Carolina, via New York, to London, Canada, over a period of three weeks in September, 1938. All the other chytrids survived this change easily. During travel they were carried in charcoal water in stoppered sterile test tubes and changed to sterile Petri dishes directly upon arrival in each place.

4. Cladochytrium replicatum Karling was most persistent. In spite of the fact that leaves containing it were discarded at once, it was very hard to eradicate from all cultures. It did, however, produce resting spores very easily, and when this happened while other chytrids were still producing active zoospores, the spores were washed off into fresh dishes, and gross cultures lacking C. replicatum were eventually established.

5. The fifth chytrid (an inoperculate form belonging to the Cladochytriaceae and called simply No. 5

in our laboratory up to date), Catenochytridium nov. gen., and Septochytrium nov. gen. (both described below) were got into the accumulation stage by varying the substrata as follows:

(a) Young wheat leaves were introduced into the mixed cultures of these growing in grass in August. The zoospores of Catenochytridium are comparatively sluggish in disposition and range of dispersal. When the wheat was added just as these zoospores were discharging, they settled upon it in great numbers, and infection occurred quickly. These leaves were at once removed, washed, and transferred to fresh dishes.

(b) Old, tough pieces of corn leaf were added to the mixed cultures in August. Septochytrium nov. gen. was able to maintain itself in these, especially near the veins, over a longer period than the others. These tough midribs with sporangia of Septochytrium were removed, washed, and transferred to fresh dishes to give the accumulation cultures.

(c) In February, 1939, leaves of rye were added to mixed cultures in which No. 5 had been growing weakly all winter. These became heavily infected, and sub-cultures showed no other chytrid present. Since then it has also been grown successfully on corn leaves.

By completely selective accumulation, by plating, or by the dilution method there is next produced a culture with only one chytrid species present. The term "unifungal," while clumsy, has been adopted for this type of culture with similar connotation to the "Art Reinkultur" of Kufferath (1929) or the "unialgal" culture of Smith (1933), Bold (1936), and others—viz., a culture containing only a single-

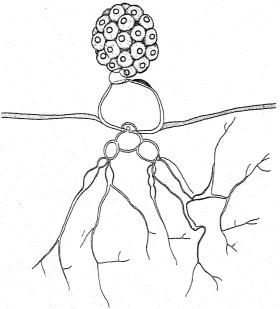


Fig. 1. Mature thallus of Catenochytridium carolinianum. The catenulate compound apophysis and the rhizoids are inside a cell of wheat leaf; the operculate sporangium with its attached spore case and the spore ball are extramatrical. Drawing made with Spencer camera lucida.

species of fungus (in this case a chytrid) although other organisms may occur. "Pure culture" might then be restricted to one consisting of only a single fungus species with no bacteria, rotifers, or other organisms present. This is the German "Absolut Reinkultur." Accustomed as we are to this in the higher fungi, there is but one record in the literature of members of the Chytridiales growing in "pure culture" for longer than a month (Couch, 1939). Sparrow (1931) obtained growth of Cladochytrium Nowakowskii on corn meal agar but not in pure culture. Butler and Humphries (1932) were successful in culturing Catenaria anguillulae, while Karling (1935) grew Cladochytrium replicatum on corn meal agar and soil mannite agar for a month before it became contaminated. Professor J. N. Couch, of the University of North Carolina, has made a "pure culture" of this last mentioned chytrid. It was secured during the summer of 1938 from an agar plate, on which a mixture of several of the chytrids from the original culture were growing, by the separation of rhizomycelium with sporangia and is, I believe, still being carried in his laboratory. He also records a form designated "Catenaria-like without rhizoids" as being in pure culture from a single spore since 1932. Four species of monocentric chytrids were carried on agar in pure culture for several generations from a single spore. The cultures were then discontinued because the "labor involved" was "excessive."

For morphologic descriptions unifungal cultures are satisfactory, and the writer has made no attempt as yet to procure "pure cultures" from them. Cladochytrium replicatum Karling, the large Cladochytrium species mentioned above, and one of the new genera (Septochytrium) have been grown on agar. Spores of the last two have been germinated on agar.

In November, 1938, several "accumulation cultures" of Catenochytridium nov. gen. seemed to be free from all other fungi. A piece of wheat leaf, taken from one of these, was separated under the binocular microscope into minute pieces containing one to several sporangia. Cultures made from these have contained only Catenochytridium nov. gen. since that time (a period of eight months). The genus is described below from this material. In August, 1938, a single spore was isolated on agar No. 12 (Leitner's agar) from an "accumulation culture" of Septochytrium nov. gen. using the method described by Couch (1939). The generic analysis is made from cultures of this single spore strain.

Catenochytridium nov. gen. (text fig. 1) is distinguished mainly by its catenulate, compound apophysis and endo-exogenous method of development, features which have proved constant; hence its generic name.

Catenochytridium gen. nov. Thallo intramatricali atque extramatricali, monocentrico, eucarpico. Zoosporangio extramatricali, operculato, pariete maturitate disjuncto. Zoosporangio evolvente endo-exogeno. Apophyside intramatricali, composita, seriebus linearibus facta segmentorum constrictorum atque catenulatorum subministrante rhizoideo systemate maxime ramoso. Zoosporis cilio pos-

teriore praeditis, cumulo globoso emergentibus quieteque aliquamdiu manentibus mox rapide atque emicatim enatantibus. Spora perdurante extramatricali, eodem loco quo zoosporangium formata, segmento apophysidis interdum in sporam perdurantem transformato; germinatione incomperta.

Catenochytridium carolinianum sp. nov. Fungus saprophyticus; zoosporangiis hyalinis, globosis, subglobosis, pyriformibus, obovatis, ovatis, ellipticis, reniformibus vel convolutis cum lobis apiculatis, 8-40  $\times$  8-75  $\mu$ . Operculo in vacuo sporangio cardinato atque persistente in loco apiculato vel subapiculato; orificio circulari, 6-20 μ dia. Apophysidis segmentis catenulatis numero 2-30, seriebus linearibus 1-4 primariae cellulae apophysidis adhaerentibus; primaria cellula apophysidis plerumque vel globosa vel ovata, 5.5-22 μ; ceteris apophysidis segmentis globosis, ovatis, ellipticis vel variatim elongatis, protoplasmatis nexu per parietem vel isthmo elongato conjunctis. Rhizoideo systemate (apophyside addita) 55-800  $\mu$  magnitudine; rhizoidibus .5-3  $\mu$  dia., minutissime ramificatis atque aliqua ex parte dichotomis. Zoosporis hvalinis, globosis, 5-6 μ, uninucleatis, cum uno globulo maxime refractivo c. 2.5  $\mu$  dia.; cilio 35-40  $\mu$  long. Zoosporis inficientibus plerumque cum pariete hospitis a latere conjunctis. Vetula capsa zoosporae plerumque in sporangio vel in spora perdurante persistente, depressa, hyalina vel sucinea-colorata crassaque, c. 8 µ dia. Cellulosa reactione in apophyside conspicua atque in capsa vetula sporae, parum conspicua in sporangii pariete, in rhizoidibus vix discernenda. Spora perdurante levi, globosa vel ovata, 8-40 μ, crassa, pallida vel atra-sucinea colorata, cum uno globulo permaximo atque strato parietali globorum minorum; germinatione incomperta.

Catenochytridium gen. nov. Thallus intra and extramatrical, monocentric, eucarpic. Zoosporangium extramatrical, operculate, delimited by a cross wall at maturity. Method of growth and development endo-exogenous. Apophysis intramatrical, compound, consisting of linear series of constricted, catenulate segments subtended by an extensive, richly branched rhizoidal system. Zoospores posteriorly uniciliate, emerging in a globular mass and lying quiescent for a few moments before swimming away; method of swimming rapid and darting. Resting spore extramatrical, usually formed in the same position as the zoosporangium or a segment of apophysis sometimes encysting and becoming a resting spore; germination unknown.

Catenochytridium carolinianum sp. nov. Zoosporangia hyaline, spherical, sub-spherical, pyriform, obovoid, ovoid, elliptical, kidney-shaped or convoluted with pointed lobes,  $8-40 \times 8-75 \,\mu$ . Operculum apical to sub-apical in position, hinged to and persistent on the empty sporangium; orifice circular, 6-20 μ in dia. Catenulate segments of apophysis 2-30 in number, arranged in 1-4 linear series attached to the primary apophysate cell; primary apophysate cell commonly spherical to ovoid,  $5.5-22 \mu$ ; other segments of apophysis spherical, ovoid, elliptical or irregularly elongate, joined by an imperceptible or an elongated isthmus. Rhizoidal system (including apophysis) 55-800  $\mu$  in extent; rhizoids from .5-3  $\mu$  in diameter, becoming very finely branched; branching somewhat dichotomous. Zoospores hyaline, spherical, 5-6  $\mu$ , uninucleate, with a single highly refractive globule about 2.5  $\mu$  in dia.; cilium 35-40  $\mu$  in length. Infecting zoospores commonly attached laterally to wall of host cell. Old zoospores case usually persistent on the zoosporangium or resting spore, slightly flattened, hyaline or amber and thick-walled, about  $8 \mu$  in diameter. Treatment with chlor-iodide of zinc producing a pale

mauve color in the sporangium wall, a deep mauve in the old spore case, a deep pinkish-mauve in the segments of the apophysis, magenta in the primary apophysate cell, barely affecting the rhizoids. Resting spore smooth, spherical to ovoid, 8–40  $\mu$ , thick-walled, light to dark brown, with one large globule and a parietal layer of smaller ones; germination unknown.

Saprophytic in leaves of wheat, corn, rye, oats, and various grasses, in Chapel Hill, North Carolina, New York City, and London, Canada.

Septochytrium nov. gen. (fig. 2) is characterized by a heavy rhizomycelium with septations, constrictions and trabeculae or plugs, and by intercalary swellings. Since, on different hosts and even on the same host, it evinced extreme variability in size and shape of sporangia, in degree of polycentricity in number of septations, constrictions and swellings, it has been given the name S. variabile and has been studied from a single spore strain only.

Septochytrium gen. nov. Rhizomycelio intramatricali, praecipue polycentrico, filamentis elongatis, cylindricis, septatis atque constrictis, incrementis intercalaribus, rhizoidibus minutissime ramosis atque variatim formatis, zoosporangiis intercalaribus atque terminalibus, operculatis, cum uno (nonnunquam compluribus) isthmo variatim elongato. Zoosporis cilio posteriore praeditis, emergentibus cumulo vel globoso vel ovato atque quiete aliquamdiu manentibus mox rapide atque emicatim enatantibus. Sporis perdurantibus terminalibus atque intercalaribus, eodem loco quo sporangia formatis vel ab incrementis intercalaribus; germinatione per porum parietis recto itinere in zoosporangium operculatum evanescenti zoosporangio simile, vel in tubulam in cuius apici contenta collecta atque septo separata ut zoosporangium fiat.

Septochytrium variabile sp. nov. Fungus saprophyticus; zoosporangiis hyalinis vel subfuscis, globosis, 4–150  $\mu$ dia. (saepe 75-150  $\mu$ , plerumque 45-60  $\mu$ ) cum papilla vel isthmo curtissimo atque latissimo, ovatis, ovalibus, late pyriformibus,  $10 \times 15 \mu$ -180  $\times$  220  $\mu$  (plerumque 100  $\times$ 150  $\mu$ ) cum isthmo 4  $\mu$ -60  $\mu$ , obclavatis vel vasculiformibus,  $2 \times 6 \mu - 35 \times 360 \mu$ , calyciformibus, irregularibus, ramosis, depressis, cum una (compluribus rarius) papilla vel tubula exeunte varia longitudine atque diametro; zoosporangiis septis maturitate disjunctis, primum pariete levi, maturitate striata vel stratificata, corrugata senente; orificio operculi circulari, 1-16  $\mu$  dia. vel subovato 4 imes 6- $6 \times 10 \,\mu$ . Zoosporis hyalinis, globosis vel ovalibus, 4-6  $\mu$ , cum uno globulo refractivo .7-3  $\mu$  (plerumque 2  $\mu$ ) dia.; cilio 30-40 μ long. Rhizomycelio crasso, extenso (20 μ-1 cm.), maxime ramoso, cum constrictionibus atque septationibus vel trabeculis vel partim vel ex toto trans rhizoides extensis; in sporangiis inserto locis 1-12; diametro loco insertionis .4-10 \mu. Incrementis intercalaribus univesicularibus, singillatim vel seriatim locatis, ovalibus,  $8 \times 10-55 \times 100 \,\mu$  (plerumque  $15 \times 20 \,\mu$ ), interdum globosis, 8-25  $\mu$  dia., fusiformibus  $6 \times 26-20 \times 80 \mu$  vel irregularibus; incremento nonnunquam aucto in zoosporangium secundarium vel tertiarium vel in sporam perdurantem transformato, nonnunquam origine primaria permaxima thalli permanente, sed interdun (ut vedetur) in cellulas duas diviso, altera in sporangium transformata vel in sporam perdurantem, altera apophyside quadam permanente plerumque vacuo vel subvacuo mycelii incremento persistente. Cellulosa reactione in parietibus sporangii, inque incrementis intercalaribus conspicua. Sporis perdurantibus pallidis vel atro-sucineis coloratis, globosis, 4-60  $\mu$ , ovatism  $4 \times 6-50 \times 65 \mu$  vel elongatis,  $10 \times 35 \mu$ ,



Fig. 2. Mature thallus of Septochytrium variabile showing polycentric nature. The large central body is a young primary zoosporangium. Radiating from it is the rhizomycelium with its swelling and secondary zoosporangia. Drawing made with Spencer camera lucida.

levibus, stratificatis, vel cum extera pariete irregulari, interdum cum uno maximo globulo refractivo cumque globulis minoribus numerosis; zoosporangiis directa germinatione sporae perdurantis formatis, globosis, pyriformibus, ovatis, etc., plerumque minoribus quam sporis perdurantibus, zoosporangiis in tubula extrema, ovatis, globosis, pyriformibus vel clavatis; tubula lata atque sacculata vel angusta, longa atque spiriformi,  $7 \times 26~\mu$ – $10 \times 450~\mu$ .

Septochytrium gen. nov. Rhizomycelium intramatrical, predominantly polycentric, consisting of elongate, septate and constricted filaments, intercalary swellings and finely branched rhizoids. Zoosporangia variously shaped, operculate, terminal and intercalary, with one (occasionally several) neck of variable length. Zoospores posteriorly uniciliate, emerging in a globular to ovoid mass and lying quiescent for a few moments before swimming away; method of swimming rapid and darting. Resting spores terminal or intercalary, formed in the same position as the sporangia or from the intercalary swellings; germination through a pore in the wall to form an operculate zoosporangium similar to the evanescent one or a tube at whose tip the contents are concentrated and cut off by a cross wall to form the zoosporangium.

Septochytrium variabile sp. nov. Zoosporangia hyaline to pale brown, spherical, 4-150  $\mu$  in diameter (often 75- $150 \mu$ , average  $45-60 \mu$ ) with a very short, broad papilla or neck, ovate, egg-shaped, broadly pyriform,  $10 \times 15 \,\mu$ -180  $\times$  220  $\mu$  (commonly 100  $\times$  150  $\mu$ ) with neck 4  $\mu$ -60  $\mu$  wide, obclavate to flask-shaped,  $2 \times 6-35 \times 360 \,\mu$ , bell-shaped, irregular, flattened and depressed with one (rarely several) broad exit papilla or neck of varying diameter and length; zoosporangia delimited by true septa at maturity, sporangial wall smooth when young, striated or layered at maturity, wrinkled when empty; orifice of operculum circular, 1-16  $\mu$  in diameter, or slightly oval,  $4 \times 6-6 \times$ 10 μ. Zoospores hyaline, spherical to oval, 4-6 μ, with a single refractive globule, .7-3  $\mu$  (usually 2  $\mu$ ) in diameter; cilium 30-45 µ long. Rhizomycelium coarse, extensive (20 µ-1 cm.), richly branched, with constrictions and septations or trabeculae extending partially or entirely across the rhizoids; inserted on the sporangia at 1-12 points; diameter at point of insertion .4-10 \mu. Intercalary swellings usually persistent as empty or partially empty enlargements of the rhizomycelium but often becoming enlarged and transformed into secondary or tertiary zoosporangia or into resting spores, occasionally acting only as a very large, primary center of organization of the thallus and sometimes seeming to divide into 2 cells one of which enlarges and becomes a zoosporangium or resting spore while the other remains as a sort of apophysis. Treatment with chlor-iodide of zinc giving a pronounced violet color in the sporangial walls and intercalary swellings. Resting spore light to dark amber, spherical 4–60  $\mu$ dia., ovoid  $4 \times 6-50 \times 65 \mu$ , or elongated,  $10 \times 35 \mu$ , thickwalled, smooth, layered, or with outer coat rather irregular, usually with one large refractive globule and numerous smaller ones; zoosporangia formed in direct contact with the resting spore at germination spherical, pyriform, ovate, etc., usually smaller than the resting spore; zoosporangia cut off at the end of a tube from the resting spore oval, round, pyriform or clavate; tube wide and saccate or narrow and long with twists and coils, 7-26  $\times$  $10-450 \ \mu.$ 

Saprophytic on various grasses, wheat, rye, oats and corn leaves and narcissus root tips in Chapel Hill, North Carolina, New York City and London, Canada.

The erection of a new genus for this fungus may be controversial. In size and appearance of zoosporangia as well as in their diversity of shape there is a similarity to Endochytrium operculatum as described by Karling (1937). However, the latter is a monocentric form. While Endochytrium ramosum Sparrow is figured as polycentric, the genus itself is characterized as lacking swellings on the mycelium. Megachytrium, a new genus described by Sparrow (1933), possesses "intercalary swellings marked off by cross walls" and "occasionally septate" mycelium. But the mycelium is "never rhizoidal." Obviously a member of the Cladochytriaceae, our fungus is perhaps most nearly related to

the operculate genus Nowakowskiella. Yet the absence of a swollen apophysis, proliferation of sporangia, and the extramatrical growth habit of the rhizomycelium characteristic of this genus militate against the inclusion of our fungus in it. Contrarily, the great size, the abundance and character of resting spores, and most especially the constricted and septate appearance of the rhizomycelium make it seem prudent for the present to adopt a new generic name.

#### SUMMARY

A new genus, Catenochytridium, is proposed for an operculate chytrid with a catenulate, compound apophysis and the endo-exogenous method of growth. The type species, C. carolinianum, occurs saprophytically in leaves of grass, and cereal grains in association with other chytrids.

A second new genus, Septochytrium, is suggested for an operculate, polycentric chytrid having a coarse rhizomycelium, with constrictions, septations and intercalary swellings. The type species, S. variabile, occurs saprophytically in leaves of grass and cereal grains. It is extremely variable in form and size.

These two chytrids have been separated from five others with which they were originally associated and subsequently cultured in "unifungal" state under as nearly sterile conditions as possible.

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# SUGAR EXCRETION IN IMPATIENS SULTANI<sup>1</sup>

Miriam G. Groner

The excretion of sugar by members of the species *Impatiens Sultani* was first noticed in plants that were kept in the laboratory for general use. As no mention of the property was found in the literature, a study was made of conditions controlling sugar excretion and of the chemical nature of the sugar.

I. Sultani has been grown extensively under glass and as a house plant since a few years after its introduction from Zanzibar by Sir John Kirk (Hooker, 1882). The species was originally described by Hooker (1882), and the first American description

was in Vick's Magazine (1884).

EXPERIMENTAL PROCEDURE.—Under experimental conditions some of the plants were grown in a greenhouse, and others were kept on the window sills of a room in which the humidity was relatively low. The size of the individual granules of accumulated sugar was greatest on plants in windows receiving sunlight, but fair accumulation was obtained even on those plants kept in north windows. In the greenhouse, accumulation was good as long as the humidity was low. In cloudy weather, however, the humidity was often too high to permit rapid evaporation of water from the droplets of sugar solution that were excreted. Even in clear weather, the hu-

<sup>1</sup> Received for publication May 22, 1939. These experiments were conducted in the botanical and chemical laboratories at Bucknell University, and this opportunity is taken to express sincere appreciation for

the use of facilities.

midity was at times too high to permit rapid evaporation. All the plants were supplied with plenty of water from below.

The plants used were all grown from cuttings of three parent plants—one with solid green leaves and rose colored blossoms, one with solid green leaves and salmon blossoms, and one with leaves variegated green and white, and rose blossoms. Sugar was first noticed on a plant of the salmon variety, which was obtained in North Carolina. The other varieties were obtained later in Pennsylvania.

If the cuttings were well rooted when potted, good sized sugar granules were usually obtained within two or three weeks in a dry atmosphere. The best yields of sugar were obtained after the plants had become well pot-bound. Usually the sugar was not collected until the leaves were ready to be dropped from the plant by abscission, for at that time the granules were of maximum size and were completely solidified. Granules on leaves that were active photosynthetically were difficult to handle, as they were frequently solid and crystalline on the surface, but liquid in the center.

Figure 1 shows a branch from a plant of the salmon variety that was grown under conditions favorable to sugar accumulation. It may be observed that the sugar is excreted at the tips of hair-like protuberances located on the sides of the petioles, and to a lesser extent from the tips of the serrations on the margins of the leaf blades. These protuberances are



Fig. 1. Branch of Impatiens Sultani showing well-developed granules of sugar (natural size).

multicellular in structure and appear to be modified leaf serrations, each with a hydathode at the tip.

The mechanism of excretion is apparently similar to that causing exudation from the leaf tips of cereals and other plants having hydathodes, but is more active as excretion occurs as long as the roots are well supplied with water, regardless of the degree of saturation of the atmosphere. Relationship to root pressure and to growth substances has not been investigated as yet.

Sugar is apparently excreted under all conditions favorable to photosynthesis but is conspicuous only under conditions favorable to its accumulation. Out-of-doors, sugar is either washed away by dew or rain as soon as it appears or is eaten or carried away by insects. At the same time that sugar is being excreted, starch is being formed in large quantities within the leaf.

Plants with leaves bearing sugar that were kept in the dark for a period of three weeks did not show any marked etiolation, although almost complete abscission of sugar-bearing leaves occurred within a few days.

The best yields of sugar were obtained in a dry atmosphere when the temperature was between 21°C. and 27°C., and the plants were supplied with plenty of water from below. Too high a temperature in a dry atmosphere seemed to retard the rate of growth, but in a moist atmosphere the plants thrived at temperatures up to 33°C., or even more. The plants did well at temperatures as low as 10°C.

For analysis the granules of crystalline sugar were collected from time to time in weighing bottles of known weight, and the bottles of sugar were kept in a desiccator until a constant weight was obtained. A separate bottle was used for each variety. Before the sugar was subjected to chemical analysis, the weight of individual granules of the three varieties was determined. The granules obtained from the variety with green leaves and salmon blossoms varied in weight from 0.0007 g. to 0.0041 g. (average of 12—0.0024 g.); those from the variety with green leaves and rose blossoms varied from 0.0006 g. to 0.0044 g. (average of 12—0.0022 g.); and those from the variety with variegated leaves ranged from 0.0002 g. to 0.0016 g. (average of 12—0.0007 g.).

Plants from which these granules were collected were kept under identical greenhouse conditions, and the selection was representative as to size. The average weight of the granules produced by the varieties with solid green leaves does not vary appreciably. However, those produced by the plants with variegated leaves are very much smaller than those obtained from either of the solid green types. In variegated plants, no sugar is excreted by pure white leaves, and most of the granules are produced by leaves having comparatively large green areas.

The figures regarding the two green types apply only under optimum conditions for sugar formation. Actually, the salmon variety produces appreciable quantities of sugar under a much wider range of conditions than the rose variety. No quantitative determinations of the amount of sugar produced un-

der varying conditions were made, as accurately controlled conditions could not be obtained.

Chemical analysis.—Sugar from all three types was tested qualitatively with the Foulger (1932) reagent containing urea. Samples from all three varieties gave a deep blue-green color on boiling with the acid reagent, indicating that the Impatiens sugar yielded large quantities of levulose on hydrolysis. This indicates that the original substance must contain a large proportion of either sucrose or inulin, and inulin is ruled out by the extreme solubility of the excreted granules in water and by additional and more conclusive evidence obtained through hydrolysis of the sugar, as shown in figure 2.

When a gram or so of sugar granules from each of the two green varieties had been collected, the bottles containing them were placed in a desiccator until a constant weight was obtained. This was necessary, as the freshly collected granules contained some water. The sugar was then dissolved in about 25 ml. of distilled water. Solid impurities, such as portions of dried petiolar hairs and dust, were removed by filtering the solution through a weighed Gooch crucible. The weight of dry impurities was deducted from the original weight of sugar before the percentage composition of the solution used in analysis was calculated. The filtered solution was diluted to a volume of exactly 50 milliliters. Approximately half of this was used to determine the specific rotation of the sugar (Rolfe, 1919), and the remainder was used to determine its molecular weight by the freezing point method (Findlay, 1926).

The rotation of polarized light by the sugar solution was measured with a saccharimeter of the Ventzke type, and the specific rotation calculated

Table 1. Specific rotation and molecular weight of sugars from Impatiens Sultani, compared with the known specific rotation and molecular weight of common sugars.

Type of sugar	Specific rotation	Molecular weight
Impatiens (rose), June, 1938	60.4	281.5
Impatiens (salmon), June, 1938 Impatiens (salmon), December,	61.4	292.3
1938	63.0	299.5
lized	64.06	
Impatiens (salmon), June, recrystallized	66.28	••••
Impatiens (mixed), before hydrolysis	61.3	
Impatiens, after hydrolysis	20.8	
Cane sugar, before hydrolysis	65.1	
Cane sugar, after hydrolysis	-21,1	
Sucrose	66.412	342
Dextrose	52,5	180
Maltose	138.48	342
l-glucose		180
Levulose		180
Invert sugar		180

by the usual formula (Rolfe, 1919). All readings, except those during hydrolysis, were taken at 20°C.

The freezing point of the sugar solution was determined by the Beckmann method (Findlay, 1926), and the molecular weight of the sugar calculated from this value.

The results of these determinations, as well as some additional ones are given in table 1. Comparable known factors for the commoner sugars, as taken from the Handbook of Chemistry and Physics (Hodgman, 1936) are also included in this table.

For hydrolysis, a solution of mixed sugar from the salmon and rose varieties of *I. Sultani* was prepared by the method described above. A solution of cane sugar of similar concentration was also prepared. Hydrochloric acid was added to the sugar solutions in the proportion of 0.25 ml. to 50 ml., so that the resulting solutions were approximately

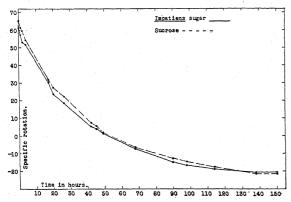


Fig. 2. Inversion curves obtained from the hydrolysis of *Impatiens* sugar, and can sugar with .05 M. HCl, at 95°C

0.05 M. for both sugar and acid. The optical activity of both solutions was measured at intervals during the period of hydrolysis, and the results are plotted in figure 2. The curves are typical of this type of irreversible reaction, in which the velocity of the reaction decreases proportionally as the concentrations of the reacting substances diminish (Findlay, 1926). The first reading was taken at room temperature, 22°C., and subsequent readings were taken at the temperature, 25°C., of the constant temperature bath in which the tubes of sugar solution were kept throughout the duration of the experiment, except for a few minutes required for readings. It may be observed that the curve for the Impatiens sugar is almost identical with that for cane sugar.

Discussion and conclusions.—The figures given in table 1, and the inversion curves shown in figure 2, indicate that the sugar excreted by *I. Sultani* is mostly sucrose, but probably contains a very small proportion of optically active impurity, possibly dextrose. Dextrose is suggested as a probable impurity because it would theoretically produce approximately the degree of error observed, and its presence would seem logical.

The values obtained for molecular weights are probably not as dependable as the specific rotations, as no attempt was made to remove soluble impurities from the original solutions. Any trace of ionizable substance of course produces a disproportionately large error in the freezing point. In determination of specific rotation, only optically active impurities are a source of error.

Values for specific rotation of recrystallized sugar very closely approach the theoretical values for cane sugar. The sugar was recrystallized by evaporating the water from the original solution in an oven at 80°C. The freezing points of the solutions of recrystallized sugar were not determined.

The sugar from the variegated plants was not subjected to quantitative analysis, as it was impossible to obtain a large enough sample within a reasonable time from the few plants available. The results from the other two varieties indicate that they both produce the same general type of sugar. Qualitative tests and physical properties would indicate that the sugar from the variegated plants is of a similar nature.

The observations reported indicate that the sugar excreted by *Impatiens Sultani* is in excess of the amount necessary either for immediate use in respiration and protein synthesis, or for storage in the form of starch. The points in favor of this conclusion are first, that the quantity of sugar excreted is generally proportional to the amount of sunlight available, and to the amount of chlorophyll present in the leaves. Second, that the greatest amount of sugar is excreted after the plants have become well potbound, at which time vegetative growth is somewhat retarded. Other factors being equal, the amount of sugar excreted was observed to vary roughly with the temperature, although there are no quantitative data on this point at present.

The plants seem to develop most luxuriantly under conditions where the excreted sugar is kept from accumulating, either by natural forces or by spraying the leaves with water. However, accumulation of sugar does not injure the plant, but merely seems to induce early abscission of sugar laden leaves. Indirectly, the plant may be injured by the numerous insects that are attracted by the sugar.

It is suggested that the excretion of sugar by I. Sultani tends to keep the concentration of carbohydrates within the plant at an optimum level. Physically the plant is of a succulent type and thrives out of doors either in full sunlight or in total shade. The plant not only grows vegetatively, but also blossoms freely in any situation where the moisture supply is adequate. The largest number of blossoms are produced in sunlight, but leaf development is at its best, and the blossoms reach their greatest individual size in partial shade, preferably with sunlight filtering through trees. Assuming that the plant is a shade plant in its native condition, the excretion of excess sugar would enable the plant to tolerate photosynthetic conditions under which more carbohydrates are produced than can be used or stored at the saturation point of the plant's tissues. This statement in no way implies that the sugar is toxic to the plant, but merely that there may be a saturation point, above which more sugar interferes with the osmotic equilibrium of the cells.

Whether or not sugar excretion occurs in other members of the genus *Impatiens* is not known, but it did occur more or less extensively in the three varieties of *I. Sultani* that were studied. In many respects this report is preliminary in nature, and the author would be interested in communicating with anyone having additional information or material.

#### SUMMARY

A solution of comparatively pure cane sugar is excreted through the petiolar hairs of the three varieties of *Impatiens Sultani* that were studied. Wherever conditions are such that the droplets of solution are not immediately removed by natural forces or artificial watering, they gradually increase in size, and lose water through evaporation until granules of crystalline sugar are formed. These granules are clean and white in appearance and have the flavor of raw cane sugar. Chemical tests—

determination of specific rotation, molecular weight, degree of inversion, and qualitative tests with the Foulger reagent—indicate that these granules are composed mostly of sucrose, mixed possibly with very small portions of dextrose. As sugar is excreted, starch is formed in large quantities within the leaves.

The quantity of sugar excreted varies with the amount of sunlight available to the plant, and less noticeably with the temperature. Varieties with leaves that are variegated green and white produce only very small quantities of sugar as compared with the commoner types with solid green leaves. The greatest amount of sugar is produced after the plants have become well pot-bound.

Although *I. Sultani* is a native of tropical Zanzibar, it has proved to be unusually adaptable to varying conditions under cultivation in the temperate zones, thriving and blooming in either sun or shade. The ability of the plant to excrete excess sugar is suggested as a possible explanation of this unusual adaptability.

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# THE ACTION OF ULTRAVIOLET RADIATION ON DERMATOPHYTES. II. MUTATIONS INDUCED IN CULTURES OF DERMATOPHYTES BY EXPOSURE OF SPORES TO MONOCHROMATIC ULTRAVIOLET RADIATION <sup>1</sup>

C. W. Emmons and Alexander Hollaender

It has been known for several years that variants can be induced in fungi by suitable exposure to ultraviolet radiation (Smith, 1936). We will present in this paper measurements of the fungicidal action of monochromatic ultraviolet radiation and the correlation observed between the systematic appearance of large numbers of variants and both the wavelength and energy of the inciting radiation. We believe that variants of the order to be described, because of their characters and permanence, repre-

<sup>1</sup> Received for publication May 15, 1939.

This material was presented in a paper at the Richmond meetings of the Mycological Society of America and as a demonstration before the Genetics Society of America, December 28 and 29, 1938. See Genetics 24: 70-71, 1939.

We wish to express our thanks to Dr. F. S. Brackett, National Institute of Health, Washington, D. C., and to Dr. M. Demerec, Carnegie Institution of Washington, Cold Spring Harbor, for careful reading and criticism of this paper. sent true genetical changes, and we shall accordingly refer to them as mutants

ingly refer to them as mutants.

The fungus used is a strain of Trichophyton mentagrophytes isolated from dermatophytosis ("ringworm") of the arm. It resembles the variety often referred to as T. asteroides (fig. 3) and is a typical dermatophyte (Emmons, 1934). In another paper (Hollaender and Emmons, 1939) we are reporting the lethal effect of monochromatic ultraviolet radiation between 2,280 and 2,950 A upon spores of another strain of the same species isolated from dermatophytosis of the foot ("athletes' foot"). Information about the relative fungicidal values of different wavelengths for fungi in vitro may lead to increased efficiency in the therapeutic use of ultraviolet.

Although the rate of appearance of mutants in pure cultures of fungi can be increased by exposure to ultraviolet radiation and other external forces such as X-rays, heat, and chemicals, mutations may

ccur independently of such influences. The sponaneous production of mutants is a prominent feaure of the dermatophytes. In some strains, if subultures are made from atypical spots which appear n the surface of old colonies, mutants differing ridely from the parent type can be easily isolated Emmons, 1932). In the present experiments we sed voung cultures in which spontaneous mutation ad not occurred. The possible significance of sponaneous and induced mutation in the classification of he dermatophytes will be discussed later.

EXPERIMENTAL TECHNIQUE.—As in the other tudies reported, the spores were suspended in a alt solution which had no measurable absorption or the wavelengths used (2,280-2,950A). The denity of the suspension was adjusted in most cases so hat there were about seventy million spores per ubic centimeter. A water cooled, high pressure, uartz capillary, mercury vapor lamp was used as he source of ultraviolet radiation. The radiation assed through a quartz monochromator, thence hrough a quartz window into the exposure cell there the spore suspension was rapidly and contantly stirred. For details of the methods used see Hollaender and Claus (1936). The density of the pore suspension insured that all radiation, direct nd scattered, was absorbed.

It was investigated separately whether or not our echnic of irradiating liquid suspensions was justied, and it was found that in the concentration used n these tests (about 70 million spores/cc.), depth f cell (2 cm.), size of cell (8 cc.), and with thorugh stirring the incident energy divided by the umber of spores will give the average energy each pore received. Details of these tests will be pubshed separately.

Before exposure 0.1 cc. of the spore suspension as withdrawn with a sterile pipette from the free rm of the exposure cell, suitably diluted in physioogical salt solution, and plated out. A control of is sort was set up for each experiment and for each ifferent wavelength in a given experiment. During ne exposure, similar 0.1 cc. samples were removed t appropriate intervals and plated out in the same lanner on corn meal agar plates. These were inibated at 30°C. or at room temperature for five or x days. The colonies which developed were counted order to determine the survival ratio. After the irvival count was recorded, all colonies from a iitably diluted plate or plates were separately subiltured to slanted culture tubes of modified Sabouud's agar.2 The transfer to slants revealed any renotypic changes which had been induced by ulaviolet radiation. Such isolation of exposed spores as necessary because most of the induced mutants ow more slowly than the normal and would have en overgrown by neighboring colonies on the origiil plate.

<sup>2</sup> The ingredients of Sabouraud's medium being unobinable, a number of peptones were tested. Neo-peptone, product of the Difco Laboratories, and C. P. dextrose ere found to be satisfactory substitutes. Some dermatolytes, however, do not grow in exactly characteristic ittern on this medium.

Production of

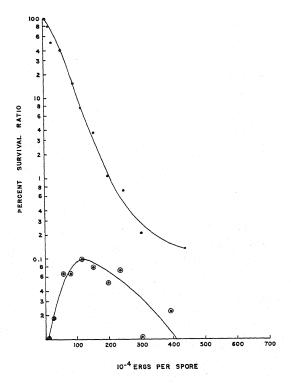
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		P	Increased	Inc	Increased	Decreased	sased	Deci	Decreased					
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Wavelengths A	Percent- Total age	nt- Total	Percent-	Total	Percent-	Total	Percent-	Total	Percent-	Total	Percent-	No.	Total picked	Percentage of mutants
080.6	6.9	0	:	19	59.4	တ	9.4	တ	9.4	5	15.6	33	1,498	2.13
2,380	5 14.3	₹	11.4	21	.09	_	9.9	₹	11.4	0	:	35	1,406	2.49
2,537	5 7.4	<b>∞</b>	12.	33	49.9	4	.9	6	13.4	œ	12.	29	2,334	2.87
2,650	15.2	5	6.3	46	58.3	CS.	2.5	6	11.4	ç	6.4	79	2,833	2.78
2,805	4 10.8	တ	8.1	08	54.	တ	6.8	9	76.9	<del>,</del> -	.27.	37	1,465	2.52
2,950	4 21.	7	5.3	11	57.9	0	:	C.	10.6	T	5.9	19	1,445	1.31
Total of type mutant	čs		31	î	150	13		33		06	-		S.	- 69
Type of mutant percentage 11.9	age 11.9		7.8	43	55.8	Ą	4.8	12.2	c;	7	.5		1(	2001

Usually 80-100 colonies from a given "run" (i.e., a planting from one exposure interval at a given wavelength) were thus transferred. This was, in effect, a random sampling of the spores which had been exposed to radiation. The number of "runs" varied with different experiments, but in some individual experiments 2,200-2,700 colonies were picked from plates and transferred individually to slants for observation of their development. In all, some 13,000 colonies, representing equivalent samples of 15 billion spores, have been so handled in obtaining the accompanying data. It was found that numbers of this magnitude were necessary in order to obtain reproducible and statistically significant results.

In studying variation phenomena one must be sure that the original material is in pure culture and that what appears to be the origin of mutants is not actually the dissociation of two unlike strains at first carried along together. In part of these experiments the plating out method was used to insure purity of the strain; but in later experiments single spore isolates were used. Previous studies have indicated that the spores are uninucleate, as well as unicellular. Single spore isolates were also made from many of the mutants when they appeared. This refinement in technique, so far as we could determine, did not alter the results.

RESULTS.—Two sublethal effects were observed. The first was a retardation of germination. This was always apparent by comparison with control plates and was checked by measurements of the germ tubes of spores made at appropriate intervals and in a suitable number of microscope fields on control and irradiated plates. This was a temporary physiological effect which disappeared on subculturing. Temporary effects of this type are not indicated on either the charts or the table accompanying this paper. To determine the mutation rate in these slowly growing spores, 425 retarded and very small colonies were picked under the dissecting microscope. Only three of these differed from the normal colony type on subculture. This appears to be a considerably lower rate of variation than was observed in random sampling. The significance of this apparent decrease in rate is not yet clear.

Besides these temporary changes, however, we observed other alterations which apparently are permanent. Permanent variations or mutations of the latter type may appear spontaneously in old cultures or they can be induced in young cultures or spores by exposure to ultraviolet radiation or other influences. One type of growth appears so constantly as a variant in old cultures of dermatophytes that, following Sabouraud, it is referred to by medical mycologists under the special name of "pleomorphism." This variant is a more or less sterile white mycelial growth which is less virulent for animals and may correspond, in a general way, to variants of some pathogens of plants in which virulence is similarly reduced. Spontaneous mutations in these fungi is by no means limited to one type of change, and if properly searched for in old cultures,



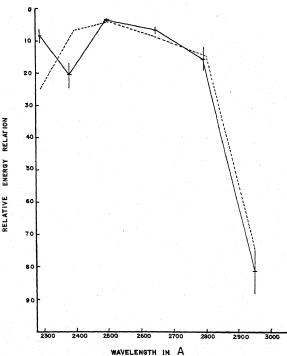


Fig. 1 (above). Lethal effect (upper curve) and production of mutants (lower curve) following exposure of spores of *Trichophyton mentagrophytes* to monochromatic ultraviolet radiation of wavelength 2,650A.

Fig. 2 (below). Wavelength dependence of lethal effect (dotted line) and mutant production (solid line) when spores of *T. mentagrophytes* are exposed to monochromatic ultraviolet radiation.

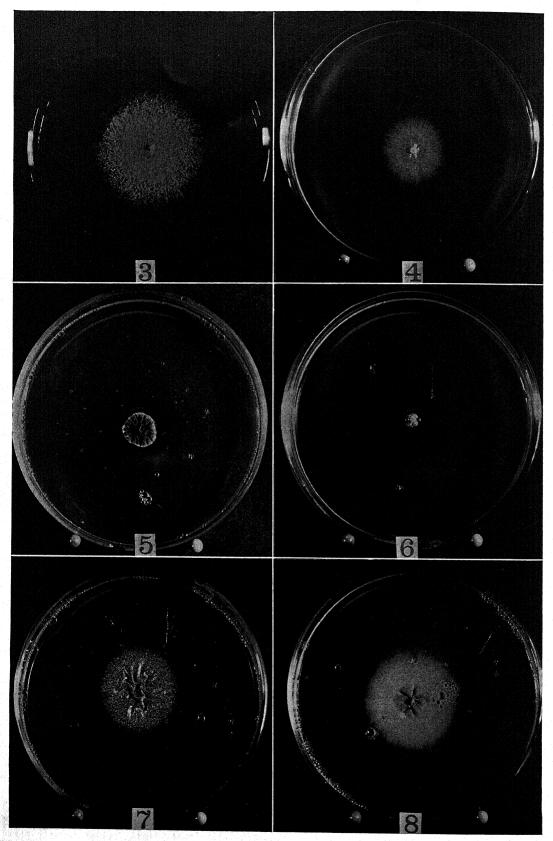


Fig. 3-8.—Fig. 3. Ten-day culture of the strain of dermatophyte used. All cultures in the entire series are of the ame age and grew under identical environmental conditions.—Fig. 4-8. Ten-day cultures of mutants of the strain hown in fig. 3, belonging in Group II.

mutants differing from the parent in colony type, color, morphology, etc., can be found and isolated (Emmons, 1932). No mutants of any type appeared in our control cultures (isolations from nonirradiated spores), which numbered over 2,000. This is explained by the fact that we took care to use only young vigorously growing cultures in preparing the spore suspensions. When, in a later experiment, subcultures were made from pigmented and otherwise atypical spots of growth on old cultures of this strain, we were able to isolate mutants resembling some of those induced by ultraviolet radiation. Some of them were widely divergent types similar to the violaceum-like mutant shown in figure 5.

The induced mutants isolated in this study differ from the parent strain, and, with few exceptions. from each other in degree or type of pigmentation, in rate of growth, or in the amount and type of aerial hyphae produced. Table 1 indicates in tabular form the number and percentage of mutants which have been isolated after exposure of spores to the different wavelengths. The table does not show the amounts of energy to which the spores were exposed, however. The quantitative evaluation of energy required is given in the section immediately following. In order to simplify the discussion, the appearance of the different types of mutants and their classification into groups will be given after the presentation of the quantitative response. It is necessary to state here only that so far as we have determined, there does not appear to be any positive correlation between either the wavelength or the amount of energy used and the resulting type of induced mutation.

Quantitative response.—The graphs in figure 1 demonstrate results obtained in a typical experiment when one wavelength band (2,650A) was used. The upper curve, indicating survival ratio, was obtained in the manner described in our previous publication (Hollaender and Emmons, 1939). The lower curve shows the number of variants produced per 100 spores surviving exposure at energy levels given along the base line. A vertical line drawn from the points on the survival ratio curve (upper curve) to the variant curve (lower curve) indicates the actual number of variants produced at

Table 2. Experimental data for curves in figure 1.

Run	Time exposure	Energy in ergs/spore	Percent- age sur- vival ratio	age
1	5 min.	7.25×10-4	81	0
2	15.5 min.	$22.7 \times 10^{-4}$	53	1.88
3	34 min.	50.2×10-4	42.5	6.5
4	53 min.	$78.7 \times 10^{-4}$	16.4	6.5
5	78 min.	$116.7 \times 10^{-4}$	7.7	10
6	101 min.	$151.7 \times 10^{-4}$	3.93	8.2
7	132 min.	$200.\times10^{-4}$	1.03	5
8	162 min.	$247.4 \times 10^{-4}$	.61	7.7
9	198 min.	304.4×10-4	.24	0.2
10	280 min.	436.4×10-4	.153	2.5

corresponding survival ratios. For detailed data on this graph see table 2. Such graphs have been obtained for six different wavelengths between 2,280A and 2,950A. However, we have not determined as many points for some of the wavelengths as are given in this graph. In each experiment testing six different wavelengths we used a single batch of spores. This is necessary because there is some variation between different batches of spores.

Knowing the energy necessary to produce the maximum number of mutants, we calculated the amount required to produce a single mutant. These values for the six different wavelengths are plotted against wavelength in figure 2. The solid line gives the relative efficiency of different wavelengths in producing these mutants. The values are from three sets of experiments covering the entire range of wavelengths. This figure also includes a curve (dotted line) showing the efficiency of killing at different wavelengths for 50 per cent survival ratio.

Description of mutants.—We have attempted, for the sake of convenience, to classify the mutants induced by ultraviolet radiation into the six groups shown in table 1. This can be done in a general way only, because of the great variety of forms observed. Some could have been placed in either of two of the groups arbitrarily established. The most obvious character is that of color. The mutants are, therefore, grouped primarily according to an increase or decrease in pigment production; secondarily, according to presence or absence of aerial hyphae and spores.

Mutants which differed only slightly from normal are included in Group I, shown in the first double column. Most of these grew at a slower rate and produced somewhat more pigment than normal, but the differences were so slight as to make it questionable in many cases whether a given strain should be set apart from the normal. Furthermore, some of these changes were not permanent, and strains originally placed in this group contributed largely to the size of Group VI, which will be discussed later. Strains characterized by a marked increase in the amount of pigment produced by the fungus were placed in Groups II (fig. 4-8) and III (fig. 9-12). They include the majority of the mutants observed. In almost all cases the pigment was of the same wine color as that produced by the normal strain, but more intense or increased in amount. In a few cases a golden pigment was produced, and, in a few, a dark diffusible pigment. The groups are separated by the degree of development of aerial hyphae, members of Group II being nearly or quite glabrous. Groups IV (fig. 13) and V (fig. 14) are characterized by decreased pigment production and differ from each other by lack or by possession of aerial hyphae.

Group VI includes those strains which have apparently reverted to normal, and it requires more detailed consideration. At the time this analysis was made, 7.5 per cent of the variants originally picked had apparently reverted. As pointed out above, most

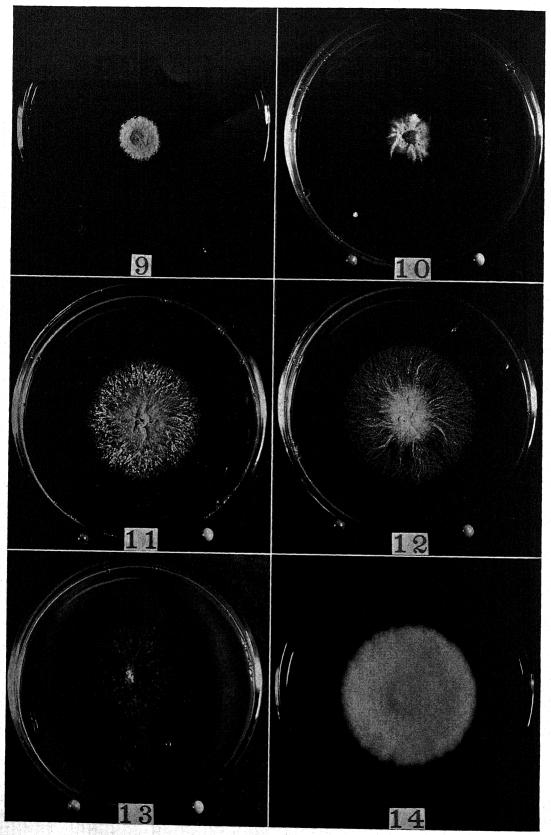


Fig. 9-14.—Fig. 9-12. Mutants of the same strain, belonging in Group III.—Fig. 13. Mutant of the same strain, belonging in Group IV.—Fig. 14. Mutant of the same strain, belonging in Group V.

of these had at first been placed in Group I because they differed only slightly from the normal. In some cases the the differences which prompted their selection were temporary, as well as slight, and probably these strains should never have been selected as mutants. There is another possible explanation for apparent reversion. In the search for mutants all colonies on a dilution plate were separately transferred to slants. It is probable that some of these colonies arose, not from a single spore, but from two or more which had clumped. If one of these spores had mutated, its growth in the heterogeneous colony would have stamped the whole colony as a mutant. However, almost all the mutants grow more slowly than normals, and in subsequent transfers of the heterogeneous colony the faster growing component (that is, the normal) would eventually be unintentionally selected, and the slowly growing mutant would be eliminated. We attempted to avoid this error by making single spore isolations of the mutants soon after their isolation. Of 52 so treated, and carried thereafter as single spore isolates, none have reverted, although two have further changed to a "pleomorphic" mycelium. A part of these 52 strains have been subcultured as many as ten times over a period of several months.

Single spore isolates of an additional series of 50 representative mutants selected at a later date and subcultured twenty times over a period of eight months have not reverted. It appears, therefore, that the mutants listed here are permanent in the same sense that the normal fungus is permanent. That is, if properly cared for, the characteristic culture and morphologic features are reproduced with fidelity in an indefinite number of subcultures, the only exceptions being further mutations which occur very infrequently in young cultures.

DISCUSSION.—It has been generally accepted that in the X-ray region increased energy will produce increased percentage of mutations, at least in higher organisms (Timofeef-Ressovsky, 1937). Our results, as illustrated in figure 1, show that with increasing energy in the ultraviolet range the percentage of mutants among surviving individuals after reaching a maximum will decrease.

The wavelength dependence curve as given in figure 2 for mutant production shows a definite maximum at 2,650A and 2,537A, where the probable error is small. The apparent decrease in efficiency at 2,380A, where the probable error is greater, may be less than is indicated by the curve. The maximum at 2,537-2,650A may be significant because it is at this wavelength that nucleic acids have their most intense absorption band (Caspersson, 1936). Proteins have a slight absorption at 2,805 but increased absorption at wavelengths below 2,400A. The nuclei of these spores are very small and do not lend themselves to cytological studies, but it has been shown that in the salivary gland chromosomes of Drosophila (Caspersson, 1936) the nucleic acids and proteins occupy definite and localized bands in the chromosomes. The greater efficiency in the 2,600 region, both in lethal and sublethal action, may indicate that the nucleic acidcontaining components of the chromosomes are most susceptible to changes and that these changes in them may effect the inherited characters of the cell. Conclusions based on such indirect evidence must be accepted with considerable caution. It is possible that other labile compounds which have the same absorption bands as nucleic acid are also present in the chromosome, but in such small quantities that they have escaped detection. It may be too that protein structures, otherwise vulnerable to injury at the shorter wavelengths, are protected by morphological structures in the cell.

The permanence, as well as the character of observed mutants, raises the question of the suitability of these fungi as material for genetical studies. Mycologists have usually avoided the word mutation for the fungi, preferring to use such terms as saltation and variation to indicate sudden changes appearing in these microorganisms in which the nuclear structure is imperfectly known. No sexual type of reproduction is known among the Fungi Imperfecti, to which group this fungus belongs. Since there is no nuclear fusion and no meiosis in the known life history of this fungus, the techniques of inbreeding, back crossing, etc., cannot be used. It is probable, however, that a genotypic alteration is immediately apparent as a phenotypic change. In the experimental work reported here, the material irradiated was a suspension of spores each of which, as indicated by earlier studies, was presumably unicellular and uninucleate. Single spore isolates derived from these uninucleate spores before radiation are identical so far as can be determined. We believe that the striking alterations in growth of some of the surviving spores following radiation represent true genetical changes.

Other explanations have been offered to account for abrupt changes of growth habit. Vegetative hyphal fusions occur in these fungi and give opportunity, if these is a heterocaryotic condition, for interchange between cells. Brierly (1929) believes that most saltations in the fungi are the recombination of characters already present. Recently Hansen (1938) described the "dual phenomenon," a term by which he proposes to designate the dissociation of two culture types which he believes are normally inherent in many Fungi Imperfecti, particularly those with multinucleate cells. His explanation of the phenomenon of variation does not seem adequate to explain the mutations we have observed induced by radiation. The conidia of Trichophyton are uninucleate, and the variants cannot be placed in two, or a few, groups. There is no reason to assume, however, that all the variation (or mutation-like) phenomena observed among fungi have one common explanation.

It does not seem practicable or necessary to describe in detail the mutants here reported. A few should be briefly noted. Several variants, similar to the one shown in figure 14, resemble the *T. inter-*

digitale, and others (fig. 9), the T. granulosum varieties of T. mentagrophytes. The strain shown in figure 5 closely resembles Trichophyton violaceum. It produces the same intense violaceous pigment; the colony is wrinkled, heaped-up, and bears few or no aerial hyphae; and the fungus grows slowly. Several mutants of precisely this type were isolated. Others closely resemble the nonpigmented phase of this same species. This does not necessarily mean that a fungus as well differentiated as T. violaceum is not of specific rank. It is a common and clinically important species in many parts of the world. However, it is not common in the United States, and its rare and sporadic isolation here might conceivably be explained by its occasional appearance as a mutant from another species. It seems apparent, from this and from other considerations, that the dermatophytes form a genetically related group of fungi; and the large number of rather poorly differentiated species in the group, the occurrence of interspecific bridging forms in many long series of isolations, and the appearance of mutants in single spored isolates, can all be interpreted as indicating that the species are highly mutable.

Strikingly different as these mutants are from the parent type, we are confident that they are actually mutants and not foreign contaminants. In the first place, strains of these types did not appear in our controls numbering more than 2,000 colonies. Second, they are obviously dermatophytes, as can be readily determined in all but a very few cases by an examination of the type of sporulation. Many of them, also, as shown by inoculation tests, are still virulent for the guinea pig, but in reduced degrees. By analogy it seems probable that some of those which appear avirulent for this animal are still virulent for man (as in the case with some other species of dermatophytes), but we have not had opportunity to test this.

Finally, in further consideration of the status of these mutants, while it is apparent that they are dermatophytes, we can also be confident that they are not strains (or "new" species) independently isolated from other cases of clinical dermatophytosis. Some of these mutants, it is true, resemble, in some respects, other strains and other species of dermatophytes. However, these latter species were not in culture in the laboratory, much less floating about in the air, at the time these experiments were being carried out, and again we can cite the fact that fungi of this type did not appear among the 2,000 control colonies isolated.

Previous studies have indicated that some mutants of this general type can be isolated from old cultures which have not been exposed to ultraviolet radiation, and the effect of such radiation seems to be to accelerate a process normally operative in these fungi. We have reason to suppose also that such mutation takes place in nature, either in the saprophytic phase of the fungus, when this occurs, or in its parasitic phase in the skin. It is, therefore, reasonable to conclude that this capacity to produce mutants explains in part at least the unfortunate multiplicity of names applied to dermatophytes.

#### SUMMARY

A method of exposing a suspension of spores of dermatophytes to monochromatic ultraviolet radiation is described. The energy was measured so that the amount expended on each spore can be calculated.

When spores of a strain of *Trichophyton menta-grophytes* are exposed in this way, besides the lethal effect, interesting secondary effects are observed, the most striking being mutant production.

Both mutant production and lethal effect are greatest after exposure in the region of wavelengths 2,537 and 2,650A.

Rate of mutations reaches a maximum at certain energy levels (in the case of these spores when exposed to radiation of wavelength 2,650A, the maximum is  $100 \times 10^{-4}$  ergs per spore) and rapidly decreases with increasing amounts of energy (increased time of exposure).

The mutants are placed in six arbitrarily delimited groups. The majority of them show an increase in pigment production and a decrease in growth rate. A few of them are indistinguishable from other species or other varieties of dermatophytes. The mutants are comparatively stable.

No mutants appeared in 2,000 control colonies isolated from nonirradiated spores. However, when spores from an old culture of the original strain were isolated, several mutants appeared, some of them being similar to those induced by ultraviolet radiation. Ultraviolet radiation merely accelerates the rate of mutation.

The significance of mutation in the taxonomy of dermatophytes is considered.

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# CARBOHYDRATE TRANSFORMATIONS IN LEAF BLADES, WITH SPECIAL REFERENCE TO SUCROSE SYNTHESIS <sup>1</sup>

# O. A. Leonard

THE PRESENT work represents a continuation of the author's previous studies on the transformation of sugars in plants (Leonard, 1938). The purpose of the present study was to investigate some factors which are important in the synthesis of sucrose. A review of the literature may be found in a recent publication by Nurmia (1935). Daoud and Tadros (1935) review the literature on the mechanisms of hexose transformations.

MATERIALS AND METHODS.—Corn (Zea mays), sorghum (Sorghum vulgare), cotton (Gossypium hirsutum), and cabbage (Brassica oleracea) leafblades were collected and experiments conducted during the summer and fall of 1938. The corn blades were gathered during August from a commercial field of hybrid seed corn at Ames, Iowa. The sorghum samples were obtained from the Agronomy Farm of Texas A. and M. College in September and October. The leaves were gathered from suckers, after the main crop had been harvested. Cotton leaves were taken from a commercial field near Bryan, Texas, also during September and October. The cabbage samples were obtained from the Texas A. and M. Horticultural Farm during November and December.

The leaves were clipped from the plants, their petioles placed in water, and removed to the laboratory. Samples were taken in duplicate and sometimes in triplicate. The sugar feeding experiments and treatment of the materials were handled as previously reported (Leonard, 1938), as were the analysis of corn blades. The sugars in cotton, sorghum, and cabbage samples were determined by a modification of the Munson-Walker-Bertrand method. Nyn's selective method was used for determining fructose (Loomis and Shull, 1937), and sucrose was inverted by invertase. The portions of the blades submerged in the sugar solutions were always discarded.

PRESENTATION OF DATA.—Influence of time on synthesis of sucrose from glucose.—Corn leaf blades were gathered at 6 a.m., August 13, 1938, from

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plants whose ears were predominately in the early milk stage. These blades were placed in quart jars containing 300 cc. of 6 per cent glucose solution in a semi-dark hallway in the basement of the botany building at Iowa State College, Ames, Iowa. Hartt (1937), Nurmia (1935), the author (Leonard, 1938), and others have found light not to be necessary for sucrose synthesis. The temperature of the hallway varied between 31° and 35°C.

Check samples were taken at the start of the experiment and after the blades had been in distilled water for 26 hours. There was a decrease of 1.20 per cent in total sugars, 1.83 percent in sucrose, and a gain of 0.63 per cent in glucose of the checks during this period. The midribs were always separated from the rest of the blades and were not included in the analysis.

The graphs of figure 1 show that sucrose increased markedly during the 26-hour period in the blades fed glucose, while the reducing sugars underwent very little change. Loomis (1935), under natural conditions, observed similar fluctuations within corn blades during photosynthesis. There was no fructose until the 14th hour, and then it was pres-

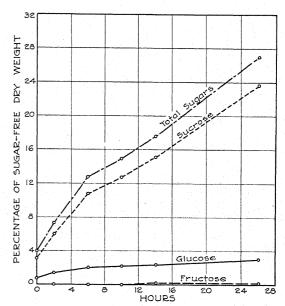


Fig. 1. Changes in the sugar content of corn blades when fed 6 per cent glucose.

ent only in traces. Evidently any fructose which may have been formed from glucose was transformed, with glucose, into sucrose. Corn leaf blades reported in a previous investigation (Leonard, 1938) accumulated more fructose than was found in the present work. The data indicate that a given sugar may be supplied to a tissue or organ and still not accumulate in the unchanged form.

Hartt (1937) found that there was a continuous increase in sucrose, but only a slight reducing sugar response from feeding leaf blades of sugar cane either fructose or glucose for periods up to 24 hours. In general her results on sugar cane were the same as are illustrated in figure 1 for corn blades. Warner (1932) fed *Elodea* leaves with glucose and sucrose and obtained fluctuations of reducing sugars and sucrose similar to those which occurred during photosynthesis.

Synthesis of sucrose from various sugars and sugar alcohols.—In general only glucose, fructose, and sucrose are found in leaves in the uncombined state. Some leaves contain sugar alcohols (Ruhland and Wolf, 1936), and these seem to be more widespread than was formerly believed. Feeding leaves sugars not found to exist in them in the free state is of interest in determining how readily these sugars are transformed into glucose, fructose, and sucrose. It is known that practically any of the sugars or

sugar alcohols will cause leaves floated in solutions of these sugars to form starch. However, all these tests have been qualitative rather than quantitative, and the possibility has not been excluded that the formation of starch may have been due to the effect of some of the sugars on the starch condensing system.

Middle-aged cotton leaves were fed glucose, fructose, sucrose, maltose, lactose, d-galactose, l-xylose, l-arabinose, cellobiose, and d-manitol. Sucrose was formed in abundance from glucose and fructose, to a lesser extent from maltose and lactose, and slightly from cellobiose. Losses in sucrose were observed when the leaves were fed l-arabinose, l-xylose, d-galactose, and d-manitol.

A more complete study of the transformation of the various sugars was made on sorghum leaf blades. For the most part the response of cotton and sorghum to these sugars was the same, and only the sorghum data will be presented. The data are shown in tables 1 and 2. As with cotton, glucose and fructose were readily transformed into sucrose. Lactose and maltose, also, formed sucrose, but the reducing sugar value was high in the lactose feeding experiment. Only a small quantity of sucrose was formed in the d-galactose feeding experiment. Lactose is a reducing disaccharide and contains glucose and galactose. Since acid and invertase hydrolysis of the

Table 1. Changes in the composition of sorghum leaf blades when fed solutions of various sugars and manitol at 34°C. for 24 hours. Data calculated as percentages of the sugar-free dry weight.

	Sucr	ose	Glucose	Fructose	Reducing sugars
Treatment	Per cent	Increase	Per cent	Per cent	Per cent
nitial composition	4.80		2.16	0.92	3.08
per cent glucose	14.55	9.75	3.25	1.20	4.45
per cent fructose		10.25	2.01	1.03	3.04
per cent sucrose	19.45	14.65	3.25	1.40	4.65
per cent lactose	9.57	4.77			7.38
per cent maltose	8.22	3.42			4.60
per cent d-galactose		0.91			13.11
per cent d-mannose		-1.20			15.10
per cent l-arabinose	3.50	-1.30	• • •	•••	12.91
per cent l-xylose		-1.10			12.10
per cent d-manitol		-3.62			3.40
Distilled water		1.94	• • •		2.70

Table 2. Changes in the composition of sorghum leaf blades when fed solutions of glucose, dulcitol, sorbitol, and cellobiose at 34°C. for 28 hours. Data calculated as percentage of the sugar-free dry weight.

	Suc	crose	Reducing	gsugars
Treatment	Per cent	Increase	Per cent	Increase
Initial composition	1.75		2.50	
6 per cent glucose	10.45	8.70	4.53	2.03
6 per cent d-sorbitol	1.09	0.66	2.31	0.19
6 per cent d-dulcitol	0.80	-0.95	2.00	0.50
6 per cent cellobiose	3.35	1.60	8.15	5.65
Distilled water	1.35	0.40	2.08	0.42

sugar extract yielded practically the same total sugar value, it is concluded that most of the lactose absorbed by the sorghum blades had been hydrolyzed. Evidently the large reducing sugar value for lactose was due to the accumulation of d-galactose, the glucose being used in synthesis of sucrose. Nurmia (1935) noted that only a little sucrose was formed in leaves of the horse bean (Vicia faba) when fed d-galactose. Leaf blades fed d-mannose decreased 1.2 per cent in sucrose during the experiment. Clements (1932) found mannose to be absent from the leaves of 42 species of plants from widely separated families and expressed the belief that mannose could not be the first sugar formed in photosynthesis.

The pentoses l-xylose and l-arabinose brought about no increases in sucrose content of the sorghum leaf blades. The sugar alcohols of d-glucose, d-mannose, and d-galactose, that is d-sorbitol, d-manitol, and d-dulcitol likewise were fed to sorghum blades, and in all cases there was a marked decrease in both total sugars and sucrose. Since galactose and mannose showed little indication of being sucrose forming, one might have expected the corresponding behavior with their alcohols, but a different behavior might have been expected with the glucose alcohol, sorbitol. Evidently none of the sugar alcohols are readily converted into their corresponding sugars, as there was always a marked decrease in total sugars when these alcohols were fed. A slight increase in sucrose was noted when cellobiose was used, although the cellobiose activity of the sorghum blades was very weak.

The reducing sugars were checked roughly to determine whether any appreciable quantities of glucose or fructose were produced from any of the other sugar forms. A mixture of yeast was added to the sugar extracts from the leaves, and these were held for 10 hours in an incubator at 34°C. From two-thirds to three-fourths of the initial reducing values remained for the leaves treated with d-galactose, lactose, l-xylose, and l-arabinose. No doubt there was some loss in the difficultly fermentable sugars, but since there was some reducing sugar (glucose) in the check, no doubt the major loss in reducing value was due to fermentation of this sugar. Nurmia (1935) obtained similar results with l-xylose and d-galactose using specific strains of B. coli in her horse bean feeding experiments.

Although several of the sugars and sugar alcohols were slightly if at all transformed into glucose and fructose in sorghum and cotton leaf blades, there is some evidence that these transformations occur in both the plant and animal kingdoms. A review of the literature on the mechanism of the interconversion of the hexose sugars may be found in a recent publication by Daoud and Tadros (1935). Daoud (1932) and Daoud and Tadros (1935) working with seeds of the fenugreek (Trigonella foenumgraecum) found that during germination the only reserve carbohydrate, a mannogalactan, was completely transformed into glucose and fructose. The mannogalactan yielded on acid or enzyme (seminase) hydrolysis in vitro equal quantities of mannose and galactose.

The sugar alcohols occur commonly in fruits and less often in leaves. Nuccorini and Bartoli (1932) thought that sorbitol was transformed into fructose and glucose in the ripening fruits of Sorbus domestica, and Martin (1937) has recently observed a similar disappearance of sorbital and accumulation of sugars with the ripening of pears.

Senescence and sucrose synthesis.—Senescence is one of the many factors which influence the transformation of carbohydrates within plants. Tables 3 and 4 show the composition of cotton blades fed

Table 3. Changes in the composition of cotton leaf blades when fed sugars at 30°C. for 24 hours September 10–11, 1938. Data calculated as percentages of sugar-free dry weight.

	Suc	crose	Reducin	ig sugars
Treatment	Per cent	Increase	Per cent	Increase
Initial composition	0.95		0.70	
6 per cent glucose	7.00	6.05	5.26	4.56
6 per cent fructose	6.06	5.01	3.41	2.71
6 per cent sucrose	7.91	6.96	3.15	2.45
Distilled water		0.60	0.60	0.10

Table 4. Changes in the composition of cotton leaf blades when fed sugars at 30°C. for 28 hours on October 1-2, 1938. Data calculated as percentages of sugar-free dry weight.

	Suc	rose	Reducin	g sugars
Treatment	Per cent	Increase	Per cent	Increase
Initial composition	0.30		2.50	
per cent glucose	2.30	2.00	16.00	13.50
per cent fructose	2.20	1.90	14.00	11.50
per cent sucrose	12.14	11.84	6.90	4.40
Distilled water	0.20	0.10	2.00	0.50

glucose, fructose, and sucrose on September 10 (before harvest) and on October 1 (after harvest). The September 10 series converted a little more than one-half of the reducing sugars absorbed into sucrose, while only about one-sixth were thus transformed on October 1. At the earlier date, the leaf blades hydrolyzed approximately one-third of the sucrose fed them. On the later date a slightly smaller portion was hydrolyzed, but the difference in hydrolysis of sucrose with age was not as marked as was the difference in sucrose synthesis. Cane sugar synthesis involves presumably, the transformation of glucose into fructose and the combination of these two sugars. Energy is involved in this synthesis, for it does not occur without oxygen (table 8). The rate of respiration, no doubt, declined with senescence and was probably an important factor in the results (Boysen-Jensen, 1912).

Percentage of water and sucrose synthesis .-Hartt (1937) and the author (Leonard, 1938) have pointed out that the percentage of water in the tissue influences the balance between the reducing sugars and sucrose. Schroeder and Horn (1922), Ahrns (1924), and Schroeder and Herrmann (1931) have shown that in wilting leaves, starch is transformed into sucrose. Schroeder and Herrmann consider that the wilting results first in the hydrolysis of starch to glucose. Glucose is then thought to be partially transformed into fructose and these two sugars combined to form sucrose. Nelson and Auchincloss (1933) and others have shown that drying potato slices at room temperature resulted in an accumulation of sucrose. Apparently potato tubers and leaves are affected in a similar manner by desicca-

Corn leaf blades were gathered at 2:30 p.m., August 12, 1938, and brought directly to the laboratory. Thirty-gram samples were placed on the laboratory benches, and the room was semi-darkened.

Duplicate samples were taken at once and after the periods indicated in table 5. Analysis of the residue showed no dextrin to be present. The data show that there was an increase in the total sugars from 2.06 to 2.50 per cent during the experiment. The increase in total sugars must have been at the expense of some acid-hydrolyzable fraction, since neither dextrin nor starch were present. No fructose was found until the third hour of drying, after which it increased until the end of the experiment. The glucose content of the blades remained almost constant. Sucrose did not show any significant change until the 9th hour of drying, after which it increased by 0.16 per cent.

Medium-aged cabbage leaves were gathered at 3 p.m. November 10, 1938, from plants which had not yet started to head, and were taken at once to the laboratory. The midribs were removed, the blades cut into strips approximately one-half centimeter wide, and samples weighed in triplicate. The latter were held at 34°C. for 18 hours, with and without oxygen. The leaves were tested with IKI and were found to contain starch at the start of the experiment, and one might expect, as others have shown (Schroeder and Herrmann, 1931; etc.), that starch would be hydrolyzed in the drying process. The blades dried at 34°C. increased in total sugars from 1.43 to 2.27 per cent when calculated on the basis of the original green weight of the tissue (table 6). Even with the large increase in total sugars, there was a decrease in reducing sugars from 1.06 to 0.24 per cent. Fructose decreased from 0.44 per cent at the start of the experiment to none at the end. This change is the reverse of that which was observed in corn blades. Glucose decreased from 0.62 to 0.24 per cent during the experiment. Sucrose increased during drying from 0.37 to 2.03 per cent.

The samples placed in nitrogen lost very little moisture. However, it is interesting to observe that

Table 5. Changes in the composition of corn blades during drying. Data as percentages of initial fresh weight.

Period of drying	$\begin{array}{c} {\rm Percentage} \\ {\rm H_2O} \end{array}$	Total sugar	Sucrose	Glucose	Fructose	Reducing sugars
0 hrs.	76.0	2.06	1.77	0.29	none	0.29
1 "	71.0	2.06	1.76	0.30	none	0.30
2 "	65.0	2.07	1.83	0.24	none	0.24
3 "	59.0	2.08	1.77	0.27	0.04	0.31
6 "	47.0	2.17	1.83	0.29	0.05	0.34
9 "	37.0	2.31	1.83	0.29	0.19	0.48
20 "	14.0	2.50	1.99	0.29	0.22	0.51

Table 6. Changes in the composition of cabbage blades stored in nitrogen or dried at 34°C. for 18 hours. Data as percentages of initial fresh weight.

	Total				Reducing
Treatment	sugar	Sucrose	Glucose	Fructose	sugars
Initial composition	1.43	0.37	0.62	0.44	1.06
Dried at 34°C	2.27	2.03	0.24	none	0.24
N <sub>2</sub> atmosphere at 34°C	1.61	none	0.82	0.79	1.61

although there was a small increase in total sugars, these blades lost all of the sucrose which they initially contained. Cabbage samples placed in a moist chamber, as will be shown later, lost only a third of their sucrose content. The necessity of oxygen for the synthesis of cane sugar will be considered under a special heading.

Another series was run on cabbage to determine the effect of moisture percentage of the blades on the synthesis of sucrose. The leaves employed in this experiment were gathered on December 19. 1938, and had been hardened by the frequent cool weather. These blades, in contrast to those harvested earlier, contained no starch. Ten-gram samples were taken in triplicate and placed in an incubator at 33±0.5°C. A check series was placed in a desiccator partly filled with water. In these the reducing sugars decreased from 2.93 to 2.41 per cent and sucrose from 0.60 to 0.40 per cent in 21 hours. Changes in the composition of the blades during drying are shown in figure 2. Total sugars, in contrast to the previous series which contained starch, decreased slightly throughout the experiment, a change which was probably ascribable to respiration. At the start of the experiment the reducing sugars were very high and sucrose low in comparison. As the blades lost water, sucrose was

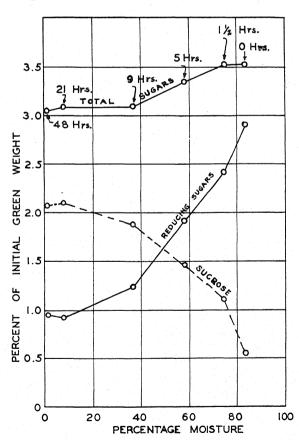


Fig. 2. The effect of drying at 33°C. on the composition of the cabbage blades.

formed from the reducing sugars. It is interesting to observe that the synthesis of sucrose continued until the blades were almost completely dry. The data confirm a previous conclusion by the author (Leonard, 1936) and by Hartt (1936) that the hydration of the tissue affects the relative proportion of the reducing sugars to sucrose. As previously sug-

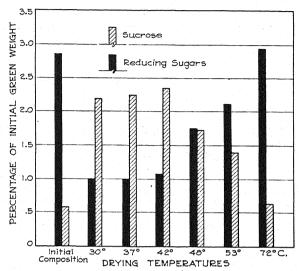


Fig. 3. Sucrose and reducing sugars in cabbage blades before and after being completely dried at different temperatures.

gested (Leonard, 1938), the reducing sugars may be confined to the vacuoles and sucrose to the protoplasm. If on drying, the vacuoles lose proportionately more water than the protoplasm, the reducing sugars will be transformed into sucrose. When leaves which had been dried were rehydrated, complete disarrangement of the protoplasm ensued, as indicated by the fact that the water in which these samples were soaked contained almost all the sugar (now completely inverted) and gave a heavy precipitate with neutral lead acetate. Evidently drying injury is due more to destruction of the protoplasmic membranes than to enzyme changes.

Temperature and sucrose synthesis.—An experiment was conducted to determine the influence of temperature on sucrose synthesis. Cabbage blades were dried at a series of temperatures, and the results are shown in figure 3. It is evident that the synthesis was undisturbed by temperatures of less than 42°C. At 48°C. the synthesis was less than at 42°C., and at 72°C. the tissue dried without change in composition. The rate of drying may have been a factor influencing these results. However, a study of the data in figure 2 reveals that the synthesis of sucrose still continued in cabbage leaf blades at the lower temperatures until they were practically dry. It is interesting to observe that even at the higher temperatures there was no hydrolysis of the cane sugar. Note also that a chemical analysis of air dried

Table 7. Changes in the composition of corn blades fed 6 per cent glucose at different temperatures. Data are percentages of the sugar-free dry weight.

Temperature	Time	Sucrose	Sucrose synthesis per hour	Synthetic efficiency	Glucose	Fructose
Initial composition.	0 hrs.	4.40			1.12	none
50°	8 "	21.83	2.18	90.0	3.06	none
0°	10 "	17.50	1.31	94.4	1.90	none
	26 "	23.76	0.74	88.6	3.60	none
8°	72 "	12.30	0.11	67.6	4.30	0.60

cabbage leaves would give an erroneous impression of the carbohydrates of the living leaf.

In a second experiment corn leaf blades were held in 6, 4, and 2 per cent glucose solutions at 50°C., 40°, 30°, and 8°C. The data for the 6 per cent glucose series are shown in table 7. The most synthesis occurred at 50°C. but the greatest efficiency was at 40°C. It is interesting to note that only at 8°C. was there any fructose detectable. Evidently the rate of sucrose synthesis was decreased at the lower temperature, or the equilibria within the tissue changed, to allow some fructose to accumulate.

The synthetic efficiency was greatest with a 2 per cent glucose solution at 50°C., being 98.7. The synthetic efficiency at 40°C. with 4 per cent glucose was only a little less than this, being 98.0. The sucrose synthesized during the experiment was almost equal to the quantity of glucose absorbed, and the corn blades contained very little more glucose at the end of the experiment than at the beginning.

Influence of oxygen and carbon dioxide on sucrose synthesis.—Many years ago Bailey (1906) noted that bananas when dipped in oil or covered with paraffin did not decrease in starch or increase in soluble sugars, while those placed in an atmosphere of hydrogen, illuminating gas, or in a vacuum, increased only a fraction as much in soluble sugars as did those exposed to the air, and lost only a fraction as much starch. Bailey later (1912) showed that ripening bananas left in air lose their starch rapidly through hydrolysis with the formation of sucrose. Boysen Jensen (1912) found that barley and pea seedlings lost some of their sucrose in an atmosphere of hydrogen and regained it again upon being returned to air. He found that the synthesis of sucrose was proportional to respiration, as measured by CO2 production. Cane sugar synthesis ceased with death, but hydrolysis continued. Nelson and Auchincloss (1933) found that slices of Irish potato tubers (Solanum tuberosum) dried in the absence of oxygen formed no sucrose, while slices dried in air accumulated sucrose at the expense of starch. They suggested that the synthesis of sucrose involves aerobic respiration. Rubin and Artsikhovskaya (1937) expressed the belief that the action of invertase in leaves is predominately synthetic or hydrolytic, depending on whether dehydroascorbic or ascorbic acid is present, and thought that this relationship explained the synthesis of sucrose by leaves in an oxygen atmosphere, with hydrolysis in an atmosphere of nitrogen or carbon dioxide.

In our experiments sorghum leaf blades were placed in a nitrogen atmosphere (oxygen removed by pyrogallic acid and NaOH) in 6 per cent glucose solutions for 24 hours. Similar series were placed in carbon dioxide at 30° and 35°C. The data for these are shown in table 8. The blades placed at 35°C. were slightly yellowed at the margin, but those at 30°C. appeared the same at the end as they did at the beginning of the experiment. It is evident that in neither nitrogen nor carbon dioxide was sucrose formed in sorghum blades. In fact, cane sugar underwent some hydrolysis during these tests and there was less at the end than there was at the beginning of the experiment. In table 6 cabbage blades in nitrogen lost all the sucrose which they initially contained. The results of these experiments verify the work of Boysen Jensen (1912) and the conclusion of Rubin and Artsikhovskaya (1937) that sucrose undergoes hydrolysis in nitrogen or carbon dioxide atmospheres. In atmospheres containing oxygen, as has been shown throughout this paper, sorghum readily forms sucrose when supplied with glucose solutions.

Other experiments were conducted to determine whether sorghum blades removed from an atmosphere of CO<sub>2</sub> could continue to synthesize sucrose.

Table 8. Changes in the composition of sorghum blades when fed glucose in  $N_2$  and  $CO_2$  atmospheres at 30°C. for 24 hours. Data are percentages of the sugar-free dry weight.

Treatment	Temp.	Sucrose	Glucose	Fructose	Reducing sugars
Initial composition		3.20	1.46	0.52	1.98
6 per cent glucose, N2 atmosphere	35°C.	1.04	15.97	2.19	18.16
6 per cent glucose, CO2 atmosphere	35°C.	1.88	9.69	1.28	10.97
6 per cent glucose, CO2 atmosphere		2.65	19.37	2.14	21.51

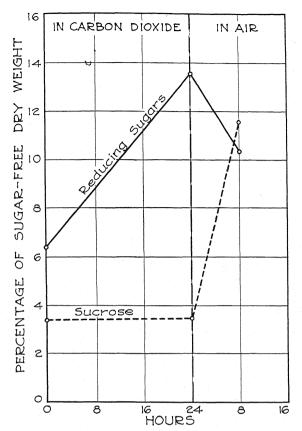


Fig. 4. Changes in the composition of sorghum blades fed 6 per cent glucose in an atmosphere of carbon dioxide and then transferred to air.

The data of figure 4 show that the sorghum leaf blades fed solutions of glucose underwent very little change in sucrose during storage in carbon dioxide. The data of table 8 show that blades stored in nitrogen lost more sucrose during storage than did those stored in carbon dioxide. The difference probably was due to the presence of some oxygen in the latter treatment which was not driven off by the carbon dioxide stream. The data clearly demonstrate that sorghum leaf blades were able to continue synthesis of sucrose after removal from the CO<sub>2</sub> chamber and that the mechanism involved in sucrose synthesis

was not seriously injured by the CO<sub>2</sub>. In this connection, it should be pointed out that the CO<sub>2</sub> contained very little water vapor, as the water was removed by CaCl<sub>2</sub>. No doubt there would have been some CO<sub>2</sub> injury had the gas been high in water vapor (Thornton, 1930).

Evidently leaf blades cannot synthesize sucrose in the absence of oxygen. In an atmosphere of nitrogen or carbon dioxide, hydrolysis, rather than synthesis, seems to prevail. Evidently sucrose synthesis is associated with aerobic respiration, as even in a zero oxygen atmosphere, considerable quantities of CO<sub>2</sub> are produced anaerobically in plants (Steward, 1937).

Iodoacetic acid and synthesis of sucrose.—Iodoacetic acid (ICH<sub>2</sub>COOH) affects certain stages in respiration and behaves as a respiratory poison. In the previous experiments it was found that oxygen was necessary for the synthesis of sucrose and presumably that synthesis is connected with aerobic respiration. Since iodoacetic acid is known to affect respiration, tests were conducted to see if it would influence the synthesis of sucrose.

The following series of concentrations of iodoacetic acid (neutralized with NaOH) were used in the glucose series; 1.0, 0.5, 0.1, 0.01, and 0.001 per cent by weight, and 0.1 and 0.01 per cent in the sucrose series. The concentrations of 1.0, 0.5, and 0.1 per cent iodoacetic acid darkened the leaf blades and seemed to show respiratory injury. Those blades placed in 0.01 per cent solutions appeared normal. The data of table 9 show that there was a loss of sucrose in blades fed 1.0, 0.5, and 0.1 per cent iodoacetic acid, but that at 0.01 per cent and less, synthesis occurred. The sucrose experiments showed a powerful hydrolysis of the sucrose when the blades were fed 0.1 per cent iodoacetic acid in solution with sucrose and still considerable hydrolysis at 0.01 per cent. It is possible that iodoacetic acid affects the synthesis in a manner similar to withholding oxygen. Again it is possible that the accumulation of certain respiratory by-products prevented the synthesis of sucrose by interfering with some stage in the process. Grant (1936) found that 0.02 molar solution of sodium iodoacetate completely inhibited the formation of lactose from glucose in slices of lactating mammary glands.

Table 9. Changes in the composition of sorghum blades when fed 8 per cent glucose solutions containing various concentrations of iodoacetic acid for 28 hours at 34°C. Results calculated as percentages of the sugar-free dry weight.

	Sucrose	Glucose	Fructose	Reducing sugars
Initial composition	4.20	5.78	2.60	8.38
1.0 per cent iodoacetic acid	2.83	28.80	5.00	33.80
0.5 per cent iodoacetic acid	3.18	31.25	5.45	36.70
0.1 per cent iodoacetic acid	3.81	35.03	2.90	37.93
0.01 per cent iodoacetic acid		11.40	3.60	15.00
0.001 per cent iodoacetic acid		6.77	3.33	10.10
Zero		6.80	2.00	8.80

KCN, NaCN, and sucrose synthesis.—Nurmia (1935) noted that additions of KCN to glucose solutions did not affect the synthesis of sucrose within leaves. She thought that since KCN affects respiration, it might also affect synthesis of sucrose. Nurmia used only one concentration of KCN, and this was not given. The writer decided that perhaps there was a critical concentration of cyanide that would be necessary to affect sucrose synthesis.

The first tests were conducted on corn blades using 0.01 and 0.001 per cent KCN. The results were essentially the same as the glucose controls. Since these concentrations were found to be ineffective on corn, the following series was used on sorghum blades: 1.0, 0.5, 0.1, 0.01 per cent NaCN. The data showed approximately the same quantity of sucrose synthesis from glucose with all the cyanide tests, even though those samples placed in 1.0 and 0.5 per cent NaCN solutions were very decidedly discolored. The cyanide solutions were strong with the odor of ammonia.

Another experiment was conducted with the hope of preventing the cyanide to ammonia reaction. A NaH<sub>2</sub>PO<sub>4</sub> buffer was used, and this was adjusted to a pH of 6.4. However, the buffer did not stop the reduction of the cyanide. It was found that KCN in the presence of glucose is changed into ammonia. In the KCN series, none of the concentrations used had any affect on the synthesis of sucrose from glucose. It is possible that the absence of a cyanide effect was due to the reduction of the cyanide to ammonia, either in the glucose solution or in the leaf tissue. It is interesting to note that sorghum blades under natural conditions contain cyanide-yielding compounds, the cyanide often being as high as 0.05 per cent of the green weight (Martin, Couch, and Briese, 1938).

Other experiments.—Other experiments were conducted to determine the effect of KSCN, ether fumes, and invertase on sugar transformations in corn and sorghum leaf blades. No significant influence was noted when feeding sorghum 0.5, 0.1, and 0.01 per cent KSCN with glucose or fructose.

Corn blades in glucose solutions were placed in an incubator containing an open Erlenmeyer flask of ethyl ether. The only observable effect was to increase the fructose fraction of the reducing sugars over the controls.

Various quantities of invertase in glucose solutions were fed to corn and sorghum blades. The lower concentrations of invertase had no influence, while the higher concentrations decreased the absorption of glucose. It is concluded that the colloidal invertase did not penetrate the membranes of the border parenchyma cells. If invertase did penetrate these membranes, it did not influence the synthesis of sucrose nor its hydrolysis, which seems improbable.

Discussion.—Corn leaf blades fed glucose through the transpiration stream increased only slightly in their glucose content but showed a marked increase in sucrose. In the synthesis of su-

crose, glucose should have been partially converted into fructose; yet fructose was not detectable until the fourteenth hour of feeding. If fructose was formed from glucose, it was transformed into sucrose so rapidly as not to be detected. Fructose might thus be formed in photosynthesis and its presence not be shown.

The observation that sucrose can be hydrolyzed to equal parts of fructose and glucose cannot, however, be taken as proof that it is synthesized from the same mixture. It is possible that sucrose was synthesized directly in our experiments from two molecules of glucose or two of fructose and that the appearance of the second hexose within the tissue was the result of sucrose hydrolysis. Sucrose increased markedly within the corn blades, while glucose increased only slightly, even though the latter sugar was the one supplied the samples. It is clear that fluctuations of sucrose within a leaf cannot be taken as proof that that sugar is the first one formed in photosynthesis. Comparable transformations might be expected with other leaf constituents. Hartt (1937) with sugar cane and Warner (1932) with Elodea have come to similar conclusions.

A variety of sugars and sugar alcohols were tested to determine how readily they are transformed into sucrose, and hence into glucose and fructose, in young sorghum and middle-aged cotton leaf blades. Of the materials seldom found in leaves, only galactose was capable of being thus transformed, and then not readily, in sorghum but not in cotton. Apparently, if any of these sugars (mannose, galactose, xylose, arbinose) or sugar alcohols (manitol, dulcitol, sorbitol) was formed in the photosynthetic process, they would accumulate as such. In view of the fact that none of them accumulates during photosynthesis and that they are nearly always absent from leaves, it is clear that they cannot be first products of photosynthesis, but are derived from secondary reactions.

Grant (1936) found that slices of lactating mammary glands when fed glucose, transformed most of the glucose into lactose. He found that active  $\beta$ -galactosidase (lactase) was entirely absent from an acetone-defatted powder of the above glands. He expressed the belief, in opposition to the classical equilibrium theory of the general nature of enzyme synthesis, that lactose could not have been formed by  $\beta$ -galactosidase. In this connection he found a greater synthesis from glucose than from equamolar mixtures of glucose and galactose, while there was almost no synthesis of lactose from galactose alone. These results are strikingly parallel with sucrose synthesis in leaves except that both component hexoses can be utilized. If lactose can be formed in the absence of the active enzyme which hydrolyzes it, sucrose might be similarly formed. Nurmia (1935) found that active invertase was almost if not entirely absent from the blades of the horse bean (Vicia faba), and yet these blades conducted a rapid synthesis of sucrose from glucose or fructose. In a previous paper the author (Leonard, 1938)

noted that synthesis of sucrose was negatively correlated with invertase. Sucrose synthesis was not believed to be due to active invertase, but to occur in conjunction with the protoplasm. It is possible that invertase "bound" with the protoplasm acts in synthesis, while "free" invertase is active in hydrolysis. Under these conditions, invertase would act as the key in the synthetic reaction, the energy being supplied through aerobic respiration. In any case it is becoming increasingly evident that active, hydrolytic enzymes do not necessarily have the powers of synthesis assigned to them by older theories.

Oxygen was found to be necessary for synthesis of sucrose in blades. Sorghum leaf blades fed glucose in nitrogen not only did not synthesize cane sugar, but lost part of the sucrose which they initially contained. Blades held in CO2 produced no sucrose, but when returned to air at the end of twenty-four hours synthesized an abundance of sucrose. Evidently oxygen or aerobic respiration is in some way connected with the synthesis of sucrose. It might be supposed that since a sucrose molecule is at a slightly higher energy level than are two molecules of glucose and fructose and since energy is released upon its hydrolysis, energy must be supplied in its synthesis. Evidently the energy supplied is obtained through aerobic respiration. Iodoacetic acid (ICH2COOH, neutralized with NaOH) reduces sucrose synthesis. Grant (1936) found that a 0.02 molar solution of sodium iodoacetate prevented the synthesis of lactose from glucose. Iodoacetic acid or its salts may interfere with some phase of aerobic respiration which is associated with synthetic reactions.

Drying of leaves, potato tubers, and probably any plant organ at normal temperatures causes the conversion of starch into sugars. In cabbage blades at the start of the drying the reducing sugars were greatly in excess of sucrose, and sucrose was formed at the expense of both reducing sugars and starch. If the reducing sugars and the invertase were in the vacuole while the sucrose synthesizing system was confined to the protoplasm, drying by reducing the relative size of the vacuole might increase the percentage of sugars within the protoplasm. The action of drying on starch hydrolysis is not understood although the observations of Scarth (1932) on reversal of starch hydrolysis in the chloroplasts are suggestive.

The following factors have been studied in their relation to sucrose synthesis: (1) Invertase active in hydrolyzing sucrose seems not to be involved in its synthesis (Nurmia, 1935; Leonard, 1938; and others). This observation is corroborated by the work of Grant (1936) on animal tissue, who found that lactose was formed from glucose in the absence of  $\beta$ -galactosidase in lactating mammary glands. (2) Chlorophyll is not essential. Hartt (1937) was able to demonstrate that albino sugar cane blades could readily synthesize sucrose from glucose. (3) Light is not necessary. Warner (1932), Nurmia

(1935), Hartt (1937), the author (Leonard, 1938), and others have found that leaves and leaf blades fed glucose or fructose in darkness form sucrose readily. (4) Oxygen is necessary. Boysen Jensen (1912), Nelson and Auchineless (1933), and the present work clearly demonstrate the importance of oxygen in the synthesis of sucrose. In the absence of oxygen, sucrose is hydrolyzed rather than synthesized, and sucrose synthesis appears to be associated with aerobic respiration. (5) The quantity of sucrose in a given organ is related to the percentage of water. Cabbage leaf blades synthesized sucrose as they dried, from the reducing sugars present. (6) Sucrose synthesis in drying cabbage blades was prevented by temperatures as high as 72°C. (7) The synthesis of sucrose continues only so long as the cells are alive (Boysen Jensen, 1912), but hydrolysis may continue if invertase is present. (8) Senility of the organ affects synthesis. Cotton blades synthesized sucrose from glucose and fructose less readily when collected late in the season after harvest than when collected before harvest.

#### SUMMARY

A study was made of several factors involved in carbohydrate transformations and in the synthesis of sucrose in corn, sorghum, cotton, and cabbage leaf blades.

Sugar changes similar to those obtained by Loomis (1935) during photosynthesis were obtained by supplying corn leaf blades with 6 per cent glucose. Sucrose increased markedly during the 26-hour feeding period, while glucose and fructose increased only slightly. Hartt (1937) has made similar observations on sugar cane blades.

Glucose, fructose, sucrose, maltose, and lactose solutions fed to sorghum and cotton blades resulted in an abundant accumulation of sucrose. Galactose and cellobiose resulted in a small increase of sucrose in sorghum. Cellobiose, but not galactose resulted in some sucrose synthesis in cotton. There was no synthesis of sucrose from d-mannose, l-xylose, l-arabinose, d-sorbitol, d-manitol, or d-dulcitol.

Sorghum leaf blades fed glucose in carbon dioxide or nitrogen lost rather than gained in sucrose. Cabbage blades left in nitrogen lost by hydrolysis all the sucrose which they initially contained.

Iodoacetic acid (ICH<sub>2</sub>COOH), neutralized with NaOH, at concentrations of 0.1 per cent or higher prevented the synthesis of sucrose from glucose, but not its hydrolysis.

Various strengths of NaCN or KCN, KSCN, ethyl ether fumes, or invertase had little effect on sucrose synthesis.

Drying corn leaf blades resulted in a slight increase in sucrose and fructose. Cabbage blades on drying synthesized large quantities of sucrose from the reducing sugars. This synthesis continued until the blades had a moisture content of 8 per cent, but was prevented by heating at 72°C.

Sucrose synthesis from glucose or fructose decreased with senescence in cotton blades.

Sucrose was synthesized less readily at 8°C. than at higher temperatures (30°-50°C.).

The synthesis of sucrose is an anabolic process requiring energy. The energy necessary is probably derived from certain stages in aerobic respiration.

Active invertase is apparently not involved in this process (Leonard, 1938).

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# A NEW SANDBUR FROM WESTERN OKLAHOMA: CENCHRUS ALBERTSONII 1

# H. Everett Runyon

On May 24, 1936, new growth of sandburs (Cenchrus) was observed emerging from the base of perennial plants on pastures near the Cimarron River north of Woodward, Oklahoma. The old burs persisting from the previous year were distinctly different from those produced by the annual, C. pauciflorus Benth., which is the only species of Cenchrus described as occurring in this locality. Similar differences with respect to the burs were observed by the author in 1935 while chartographing native vegetation in northwestern Oklahoma. Plants were transferred to the grass nursery at Hays, Kansas, in 1936 and to the breeding nursery at Woodward, Oklahoma, in 1937 where they were studied intensively during those years.

During the growing season of 1937 plants of this perennial form, Cenchrus albertsonii, were transferred to the nursery. Every month during the winter, two of these plants were transferred to the greenhouse. These plants resumed growth and flowered, also the plants remaining outside resumed growth and flowered.

BRIEF LATIN DESCRIPTION.—Cenchrus albertsonii Runyon, sp. nov., perennis, C. paucifloro annuo similis; culmi tenuiores, foliis angustioribus, spinis paucioribus reflexioribus; spiculae involucri ternae.

Herba condensa, culmis 20-70 cm. longis. Foliorum laminae juventute plicatae, senes aliquid planae, subtus carinatae, 5-15 cm. longae, 3-8 mm. latae. Spicae 4-8 cm. longae. Involucra 10-18 cm. trans spicam, spinis exclusis 3-6 mm. lata et 4-6 mm. longa, pubescentia. Spinae 20-30, extendentes, planae, basibus latiores, mediis curvatis, infimis brevioribus et minus regidis, saepe 5 mm. longis. Spiculae 2-3, saepius tres in utroque involucro.

Appellatus ex Dr. F. W. Albertson, Professore Botanico, Fort Hays Kansas State College, Hays, Kansas, U. S. A.

Description. — Cenchrus albertsonii Runyon. Perennial, resembling plants of the annual C. pauciflorus of the same region, usually the culms more slender, blades narrower, burs less crowded on the spike, the spines on the bur more reflexed. Spikelets usually three per bur, as compared with two in C. pauciflorus. Differs from C. gracillimus in its

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During the course of this work the author was associated with the Division of Forage Crops and Diseases, Bureau of Plant Industry, Southern Great Plains Field Station, Woodward, Oklahoma.

spreading culms and pubescent burs and from *C. incertus* in having spines covering the body of the bur and in being deeply cleft on the outer surface, exposing the spikelets.

Culms 20-70 cm. long, spreading, ascending or rarely suberect. Often forming dense clumps, freely branching, flattened near the base, scabrous below the spike, internodes glabrous. Blades folded when young, becoming somewhat flattened with age, strongly keeled on the lower surface rough on the upper surface, with a fine white margin. Sheaths mostly shorter than the internodes, margin scarious, loose. Ligule a ring of hairs about 0.5 mm. long. Spikes terminal, numerous, 3-10 cm. long of 10 to 18 burs, the axis flexuous, angled, scabrous, the burs 3-6 mm. wide (excluding the spines), on a peduncle about 2 mm. long, deeply cleft, exposing the spikelets, the spines 20-30, 4-6 mm. long, minutely pubescent, generally reflexed, flat, broadened at the base, the lowermost shorter and more slender. Spikelets 2 or usually 3, 4-7 mm. long, about 2 mm. wide, first glume narrow, reduced, not more than onethird the length of the spikelet, 1-nerved; the second glume slightly shorter than the sterile lemma, sterile lemma and second glume 3- or usually 5-nerved, enclosing the acuminate-pointed fruit.

Loose sandy pastures and roadsides, Harper and Woodward Counties, Oklahoma. Flowers from spring to late fall.

Named in honor of Dr. F. W. Albertson, Professor of Botany, Fort Hays Kansas State College, Hays, Kansas.

This species seems to be confined to a limited area in northwestern Oklahoma. Observations indicate that the plants are capable of withstanding moderate and dry winter but seldom retain life for more than a few years, as the old crowns are not strong as compared to other perennials.

The type specimen No. 200 collected at Woodward, Oklahoma, July 4, 1937, is deposited with the United States National Herbarium, Washington, D. C. Other specimens, No. 201 and 202, have been deposited in the Bartholomew Herbarium, Fort Hays Kansas State College, Hays, Kansas.

Thanks are extended to D. A. Savage, Agronomist, Division of Forage Crops and Diseases, Bureau of Plant Industry, for assistance with plants.

Soil Conservation Service, Marysville, Kansas

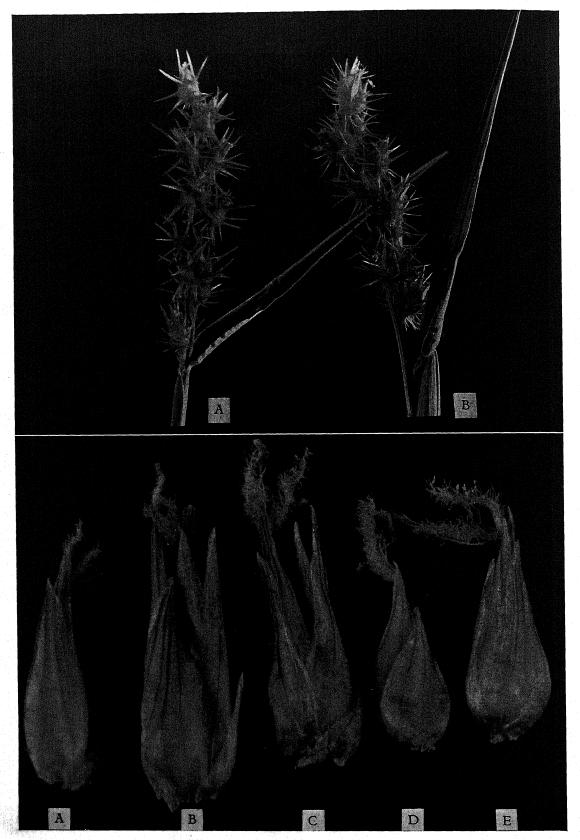


Fig. 1 and 2.—Fig. 1 (above). A. Spike of Cenchrus albertsonii. B. Spike of Cenchrus pauciflorus.—Fig. 2 (below). A and B. Spikelets of Cenchrus pauciflorus. C, D, and E. Spikelets of Cenchrus albertsonii.

# CELL SHAPE STUDIES IN THE PITH OF EUPATORIUM PURPUREUM 1

James W. Marvin

For more than a hundred years the cell has been recognized as the unit of organization of multicellular plants and animals. The structure and functions of cells have been intensively studied, but their shape as they occur aggregated into tissues has received little careful attention since it was first described as polyhedral in the seventeenth century. With the exception of the penetrating investigations by Lewis, no real attempts have been made to determine the three-dimensional form of massed cells. Additional knowledge of the shape of such cells should prove useful in the further evaluation of the factors concerned in shape determination. One hundred pith cells of Eupatorium purpureum L., favorable material because of large and relatively uniform size, were modeled on a large scale. The number of faces on each was then determined, and comparative studies were made of the areas of the faces and of the total surface areas and volumes of the cells. Such investigations add to the very limited precise data on cell shapes, and they also form the basis for an analysis and understanding of the factors underlying cell shape determination.

LITERATURE REVIEW.—As long as the existence of cells has been known, there has been speculation as to their form. Even Hooke (1665) likened the cells of bottle cork to the hexagonal compartments of the honeycomb. Grew (1675) observed that cells from the stem of borage and thistle may be pentagonal, hexagonal, or heptagonal in section, and later (1682), he stated that these "bladders" are often spherical but that in the parenchyma of the bark their shapes are similar to those of the "froth of beer or eggs." Malpighi (1687) also showed figures of parenchymal cells as angular structures.

More than one hundred years later, Sprengel (1802) observed that certain cortical and pith cells were hexagonal in outline, and he suggested economy of space (which he also assigned as the reason for the hexagonal nature of the bee cells) as the explanation. Buffon (1753) had previously considered the shape of the chambers in the honeycomb, using as an analogy the shape of peas which he caused to swell in a glass vessel. Treviranus (1806) and Rudolphi (1807) also recorded the frequently hexagonal outline of parenchymal cells.

Numerous authors have described cells as polyhedra having twelve facets arranged in various patterns. Mirbel (1801) figured the cells in the thallus of *Fucus* as hexagonal in transverse and longitudinal section. The following year (1802) he drew twelvesided parenchymal cells in three dimensions: he illustrated a rhombic dodecahedron and a modifica-

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tion of it, an elongated form with four hexagonal and eight quadrilateral faces. He also drew a double hexagonal pyramid. Although Mirbel did not discuss these figures, Bernhardi (1804) accepted the rhombic dodecahedron as one of the shapes assumed by parenchymal cells, and Kieser (1815) stated categorically that this is their form, since spheres compressed in the most economical space-filling arrangement yield this figure. A few years later he reiterated this conclusion (Kieser, 1818), and a consideration of possible shapes, including the pentagonal dodecahedron, left him convinced that the rhombic dodecahedron is the fundamental shape of parenchymal cells; he considered this the figure with the minimum surface which will stack without interstices. He pointed out that a modification of this is commonly achieved—the same modification of the rhombic dodecahedron that Mirbel represented. Kieser's views were accepted by many investigators (Meyen, 1830; Slack, 1832; Schleiden, 1845; Unger, 1846; Oken, 1847; Gray, 1887; McNicoll, 1929; and others). Link (1824), DeCandolle (1827), and Le Maout (1844) accepted Kieser's views with some reservation, since they noted that cells in section are often pentagonal or heptagonal as well as hexagonal. Von Mohl (1852) conservatively cautioned that since cells are usually of unequal size, the rhombic dodecahedron is infrequently realized in cellular tissue. De Jussieu (1852) proposed still another twelve-sided figure, a combination of a tetragonal prism with a tetragonal bipyramid (two tetragonal pyramids placed with their bases on opposite sides of a rectangular prism). He pointed out that in tissues the geometrically regular figure is seldom seen.

Bernhardi (1805) seems to have been the first of the early authors to consider cells fourteen-sided; he rejected the rhombic dodecahedron as a fundamental shape in favor of a truncated form of Mirbel's double hexagonal pyramid. Such a figure has fourteen faces. This was accepted by Kieser (1812) with a reservation, since these figures will not aggregate without interstices, and for this reason he was unable to explain their occurrence in tissues. The next year, as already noted, he changed his views in favor of the rhombic dodecahedron. Turpin (1829) also figured an unstackable fourteen-sided cell, unfortunately without discussing it, and seventy-two years after Bernhardi, Duchartre<sup>2</sup> (1877) proposed another fourteen-sided figure as the fundamental shape of parenchymal cells. This figure, like Bernhardi's, is a double hexagonal pyramid truncated at the ends. Duchartre did not describe the position and nature of the faces with reference to each other, but on the basis of his drawings of cells,

<sup>2</sup> Lewis, in a private communication, has pointed out that the passage referred to here also occurs in the first edition, 1867.

the figure seems to differ from Bernhardi's in the position and length of the lateral faces. A long and a short, apparently rectangular face appear on each side of the hexagonal cylinder alternating in their position around the figure in such a way that trihedral rather than tetrahedral angles occur.

Early investigators attributed the polyhedral form of parenchymal cells to the effect of mutual pressure on spherical structures. The importance of surface tension in such semi-liquid systems was not emphasized until Plateau published his experiments on the statics of liquids in 1873. Berthold (1886), and Errera (1886), applying Plateau's generalities to cellular systems, defined the position and shape of new cell walls, and Roux (1897) found that cleavage patterns in animal eggs were closely simulated in oil droplets. Thompson (1917) has treated extensively the relation of surface tension to cells and cellular tissues. More recently Thomson and Hull (1934) and Welch (1935) have presented Thompson's views on the position of new walls in dividing cells. Wodehouse (1935), in discussing the effect of gravity on symmetry, stated that pollen grains, on which gravitational influence is much reduced, are basically spherical, since surface tension has become much more important as a form determinant. Seifriz (1930), in a paper on the alveolar structure of protoplasm, is of the opinion that the alveoli are rhombic dodecahedra.

Previous to 1887 the rhombic dodecahedron had been considered the most economical surface-volume figure permitting the division of space without interstices. In that year, Lord Kelvin, after experimenting with Plateau's cubic frame, proposed a new fourteen-sided figure. He showed that this figure, which he later (1894) called the orthic tetrakaidecahedron, with its eight hexagonal and six square faces, dihedral and trihedral but no tetrahedral angles, is more stable than the rhombic dodecahedron, and (according to Lewis, 1925) it is also more economical in its surface-volume relationships. The orthic tetrakaidecahedron is not unknown to the crystallographers, for it was described by Romé de l'Isle (1783) as a truncated regular octahedron, by Mallard (1879), and by Tutton (1911), among others, as one of Von Fedorow's five parallelohedra, a cube truncated by an octahedron. Although he is not the first to suggest it, Baitsell (1937) emphasizes the crystalline structure of protoplasm, and he thinks that death is a crystalline pattern permanently set. Wrinch (1937) concludes that protein molecules are fundamentally hexagonal in shape and that the wide range in the number of types of proteins results from combinations of this pattern.

Matzke (1927) has shown that orthic tetrakai-decahedra of uniform size will aggregate to fill space completely. He has also described a simple method for the construction of accurate models of this figure (1931). Models, constructed by this method, were stacked (Marvin, 1936) and a formula developed to express the aggregation series. Recently Glaser and Child (1937) suggested a relation between the or-

thic tetrakaidecahedron aggregation series and animal growth by substituting this series for one of the variables in Huxley's relative growth formula. although, in another paper (1938), Glaser points out that the implications of this relationship are not clearly understood and it may be fortuitous. Hancock (1938) derived mathematically a fourteensided figure having eight triangular and six square faces with twelve vertices at each of which four faces meet at a point. Thompson (1917) was impressed with the fact that spheres of uniform size when most compactly arranged yield rhombic dodecahedra upon compression. He says that orthic tetrakaidecahedra could be realized if the walls of the spheres or cells were perfectly elastic. He thinks, however, that since cell walls are not perfectly elastic, the fundamental shape of parenchymal cells probably is the rhombic dodecahedron.

There appeared in 1923 the first of a remarkable series of studies by Lewis on the three-dimensional shape of cells. In the first paper (1923), 63 cells of elder pith were modeled from serial sections, and they had an average of 13.96 faces. Examination of 37 additional cells did not change the average. After careful study, Lewis became convinced that these cells approximated orthic tetrakaidecahedra, although he did not observe such a figure. Pentagonal faces were relatively common in addition to the hexagonal and quadrilateral faces expected. He suggests that differences in the volumes of adjacent cells might well cause the appearance of two pentagonal faces instead of the expected quadrilaterals or hexagons. The modifying effect of cell division is also considered by Lewis to be an important factor in inducing the observed irregularities. The possible effect of division on the shape of a dividing cell is discussed in detail. Lewis (1925), continuing these studies, examined 100 stellate cells of Juncus, 100 human adipose tissue cells, and 50 stratified oral epithelial cells. As in the elder pith studies, he concluded that fundamentally they are derived from the orthic tetrakaidecahedron. In another paper (1928a), the columnar epidermal cells of cucumber were found to be essentially hexagonal prisms, but with four basal contacts and one exposed surface, making eleven faces in all. He extended this study (1930) in a discussion of the effect of growth and division on the shape of these cells. A discussion of the properties of a mosaic of polygons (Lewis, 1931) and of epidermal mosaics (Lewis, 1933a) was followed by a general discussion of cell shape (Lewis, 1933b). From a study of models (1935) he showed that the tracheids of pine may also be derived from the orthic tetrakaidecahedron, although in development additional contacts occur, increasing the number to twenty or more for a mature cell.

Gray (1931) has discussed the work of Thompson, Lewis, Matzke, and others on cell shape, and he emphasizes the fundamentally biological nature of the problem. Hein (1930) sectioned the pseudoparenchyma from the sclerotia of an ascomycete in three planes at right angles to each other and found

that the cells were essentially isodiametric and hexagonal in outline. From this he concluded that they are tetrakaidecahedra and that the orthic tetrakaidecahedron is their fundamental shape.

Lewis' studies were in the main on mature tissue. Tupper-Carey and Priestley (1924), in an investigation of meristematic tissue, macerated cells from the apical meristem of a Vicia faba root. These were polyhedra, and the authors believed them to be fourteen-sided, although counts of the numbers of faces on individual cells were not made. Priestley (1928, 1929), discussing the nature of meristematic cells, emphasized the effect of internal pressure on the form of these plastic cells, and he concluded that they approach "the shape of the dodecahedron or tetrakaidecahedron." He has also considered the question of sliding growth as it may affect the form of meristematic cells (1930). An alternative hypothesis is advanced whereby cells might change their position with reference to adjacent ones without assuming a movement of one wall over the surface of an adjacent cell. This change in the relative position might be accomplished, he suggests, by a gradual mutual adjustment of the cells as a "common framework."

Gane (1930) described a method for simulating the shape of meristematic cells: plasticine balls were compressed so as to eliminate all the spaces between them. He found that when they were stacked so that twelve balls were in contact with a central one, fourteen and not twelve faces were formed. This is not in agreement with recent work on lead shot (Marvin, 1937, 1939) in which lead balls of uniform diameter were stacked in a cylinder in such a fashion that, except for the peripheral layers, twelve shot were in contact with a central one, and after compression, dodecahedra were formed. If the shot were poured at random into the cylinder and compressed, the average number of contacts was fourteen (14.17). The formation of rhombic dodecahedra or orthic tetrakaidecahedra by compression of spheres depends upon arrangement and uniformity of size.

Matzke (1939) has recently demonstrated the controlling effect of size differences on the shape of compressed spheres and its importance in cell shape determination. He compressed lead spheres of two diameters, one twice that of the other. When these shot were mixed in differing proportions, the average number of faces on the small spheres varied from 9.5 to 13.3, while the large shot had anywhere from 19.98 to 30.18 as the average number of contacts. The small shot always had fewer than fourteen contacts and the large shot more than fourteen. Correlated with these changes in the number of faces was a change in the occurrence of the kinds of faces. An average of fourteen contacts can be predicted, therefore, only when the compressed spheres are of uniform size or are in certain definite proportions.

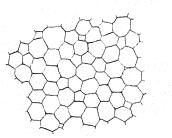
Method.—Stems of Eupatorium purpureum L. were collected in the vicinity of New York City August 9, 1937. The plants were entering the flowering

period, and there was very little vegetative growth. The pith cells from the upper quarter of the stem were large, approximately isodiametric or slightly elongated, and the intercellular spaces were small and infrequent. Hand sections of the stem made possible the selection of portions in which the pith was approximately twenty-five cells in diameter, a ratio not uncommon in stems, and approaching the ratio used in the study of compressed lead spheres in previous investigations by Matzke (1939) and Marvin (1939). Models of 100 cells from these selected sections were constructed by the method previously described (Marvin and Matzke, 1939).

The first 50 cells were taken from the more peripheral part of the pith, although the several outermost layers of pith cells were not used at all. The 25 cells modeled first were selected at random, whereas cells 26–50 were taken from a small group removed en masse. Cells 51–100 were from a fragment of the central portion of the pith.

The camera lucida drawings of the faces of the cells from which the models were constructed were made at a magnification of 170 diameters. Surface areas of the faces and of the models were measured on the camera lucida drawings with a planimeter. The models were water-proofed with a cellulose acetate preparation and their volume obtained by suspending them in distilled water from one arm of a torsion balance. To the lifting power of each its weight in air was added, thus giving the weight in grams of water and consequently the volume in cc. of the model.

Data.—Text figures 1 and 2 illustrate the appearance of the pith cells studied in transverse and longitudinal section respectively. In figure 1 the cells appear essentially isodiametric, although they vary somewhat in cross-sectional area and show 4–8 contacts. No tetrahedral angles are present in this view, and the few intercellular spaces are small triangular areas. In longitudinal section the cells have a different appearance (fig. 2). In this figure they are oriented in rows which correspond to the vertical rows in the plant (fig. 2, A, B, C, D, E, F, and G). The members of some rows are elongated (A and D), while in adjacent rows the cells are isodiametric or even flattened (fig. 2, B and C). The elongated cells have their long axes parallel to the long axis of



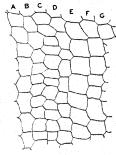


Fig. 1-2.—Fig. 1 (left). Transverse section through pith of *Eupatorium purpureum* L.—Fig. 2 (right). Longitudinal section through same. ×46.

the stem. From this view we see that the cells commonly have one upper and one lower contact. There are frequently two lateral contacts on each side for the elongated cells and one or two for the shorter ones. These lateral contacts are frequently asymmetrically placed with reference to the midpoint of the length of the cell. The transverse view gives an approximation of the number of sides, each of which may consist of one or more faces. The average number of contacts on the cells shown in figures 1 and 2 may be computed by a method developed by Lewis (1928b), similar to Duchartre's approach. There are fifty-five cells shown in transverse section in figure 1 with a total of 330 edges. They thus have an average of 6.0 sides. In longitudinal section, as seen in figure 2, in addition to top and basal contacts, from one to four lateral contacts occur. By actual count there are 108 sides with 219 lateral contacts, or an average of 2.03 lateral contacts per cell. Assuming that the transverse and longitudinal sections are typical, an average cell would have six sides with 2.03 faces on a side or 12.18 facets. Two additional contacts, one at the top and one at the base of the cell, upper and lower contacts in figure 2, increase the average to 14.18. Such an average if based on large numbers is a useful check on the more accurate study of models of individual cells.

The variations in size and form of the 100 cells modeled are shown in plates I and II, figures 1–100, which correspond to cells 1–100 in table 1. These are photographs of the models of the cells somewhat reduced in size and numbered from 1–100 in the order studied. Considerable variation in size is apparent. The model of the smallest cell, number 80, has a volume of 1.5 cc., while that of number 73, the largest, is 22.5 cc. Great diversity of form is also apparent. Cell models 53, 62, 65, 76, and 83 appear as fundamentally several-sided prisms with one or sometimes two faces on each side. This pattern, less clearly seen in many other cells, seems entirely lacking, for instance, in number 40.

The numerical data are given in detail in table I and in summary form in tables 2, 3, and 4. In table I the frequency of occurrence of each kind of face is recorded for each cell and also the total areas of each kind of face on the model. The total number of faces, the total area of the faces as measured in square inches and computed in square centimeters,

and the volume of each model are also given. In addition, the surface area of a sphere having a volume equal to the observed volume of the model was computed. Lewis (1925) has published the results of Graustein's computations in which the surface of a rhombic dodecahedron is given as 1.1050 times the surface of a sphere of equal volume, and the surface of an orthic tetrakaidecahedron is given as 1.0987 times that of a sphere of equal volume. These values were used in computing the surface areas of rhombic dodecahedra and orthic tetrakaidecahedra with volumes equal to the volume of each cell model. Thus, model number 1 has five quadrilateral faces with a combined surface area of 0.62 sq. in., two pentagonal faces whose areas equal 0.74 sq. in., and six hexagonal faces with a total area of 2.55 sq. in. The model was thirteen-sided with a total surface area of 3.91 sq. in. or 25.22 sq. cm.; its volume was 9.30 cc. A sphere of this volume has a surface area of 21.24 sq. cm., and an orthic tetrakaidecahedron and a rhombic dodecahedron of the same volume, 9.30 cc., have surface areas of 23.34 sq. cm. and 23.47 sq. cm., respectively. The sphere is the most economical surface-volume configuration, and in this series the orthic tetrakaidecahedron comes next, the rhombic dodecahedron third, with the cell model slightly less economical than the rhombic dodecahedron. At the bottom of the table appear totals of the number and the areas of each kind of face as well as the total number of faces on the 100 cells; the total area and volume of the cell models and the total computed areas of the spheres, orthic tetrakaidecahedra, and rhombic dodecahedra are also given.

The distribution of the number of contacts observed on the 100 cells is shown in table 2. The number of sides ranges from seven to twenty with no eight-sided cells observed. This is similar to Lewis' (1923) results in a study of elder pith cells in which he observed a range from 6- to 20-sided cells which included no nine-sided ones. He found 21 cells having 13 sides, the group of greatest frequency, 19 fifteen-sided, 16 fourteen-sided ones, 10 with 16 sides, and 8 each of 11- and 12-sided cells. In the present study, 12-sided cells are most common, 18 in all; those having 14 and 15 sides are next most common with 15 of each; and there are fourteen 13-sided cells, and 13 which have 11 sides. While there were more 12-sided cells than any other

Table 2. Distribution of the number of contacts per cell on 100 cells, and on the same 100 cells when divided into two groups by volume.

A. Distribution of the number of contacts per Number of contacts per cell		* ^				14 15	15 15	16 7	17	18 4	19	20
B. Distribution of the number of contacts per Number of contacts per cell	cell	in	78 c	ells (	(volu	ıme	less	than	12	cc.)		
C. Distribution of the number of contacts per c Number of contacts per cell	ell	in 2	2 cel	ls (v	olun	ne m	ore	than		·		20 1

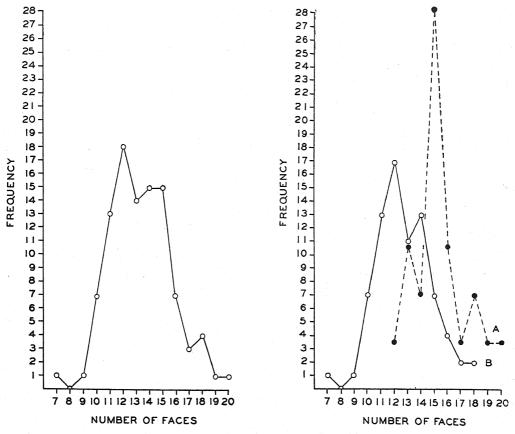


Fig. 3-4.—Fig. 3 (left). Frequency curve showing the distribution of the number of contacts per cell for the 100 cells studied.—Fig. 4 (right). Frequency curves showing the distribution of the number of contacts per cell on the 100 cells divided into two groups according to volume; curve B represents the small cells, and curve A the large cells.

kind, it is obvious that there was no polyhedron that was much more abundant than all the others. The maxima were in the range from 11- to 15-sided cells, with none markedly predominating. Thus the data on the frequency of occurrence of the number of faces observed on the *Eupatorium* pith cells show marked similarity to those recorded by Lewis for 100 pith cells. The data given in table 2A are presented graphically in text figure 3. No well-defined mode is apparent, although 12 is clearly the group of greatest frequency.

The 100 cells studied were then divided arbitrarily into two groups according to the volumes of their models (table 2, B and C): the volume of the smallest cell model is 1.5 cc. and of the largest, 22.5 cc.; accordingly, all the cells whose models have volumes of 12.0 cc. or less were grouped together, and those with volumes of more than 12 cc. were placed in a second group. Of the 100 cell models, 78 are in the first (12 cc. or less in volume) and 22 are in the second group (more than 12 cc. in volume). A distribution curve of the number of faces on the cells was plotted for each group (text figure 4). To make the curves comparable, each value for the cells having models with volumes of more than 12 cc. was multiplied by 78/22. The group of small cells has

a range of 12, from 7- to 18-sided with the 12-sided ones most common, 17 in number. The larger cells show a range of 9, from those with 12 to those with 20 faces, and the 15-sided ones, of which there are 8, are most common. It is apparent here that when the cells are divided into two groups according to the volumes of their models, a distribution curve for each shows a well-defined modal class which also rather closely approximates the average for each group. The 78 smaller cells average 12.78 contacts and the 22 larger ones 15.41 contacts. By contrast, the graphical treatment of the 100 cells as a unit (text figure 3) shows no pronounced modal class, and the group of greatest frequency, 12, does not represent the numerical mean, 13.36. This indication of groups in the population is important and agrees closely with lead shot data previously obtained (Matzke, 1939).

The division into two groups is distinctly arbitrary, and the cells may also be divided into groups depending upon the number of faces. Thus from table 1 it is possible to calculate that there are 7 cell models having 10 faces and an average volume of 4.98 cu. cm.; there are 13 models with 11 faces and an average volume of 6.48 cu. cm.; there are 18 models with 12 faces and an average volume of 7.90

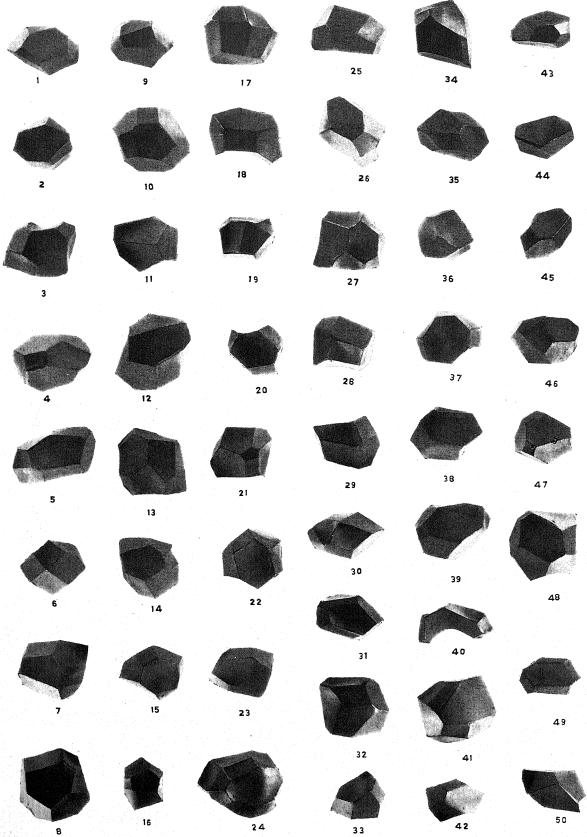


Plate I. Models of cells 1-50. ×85.

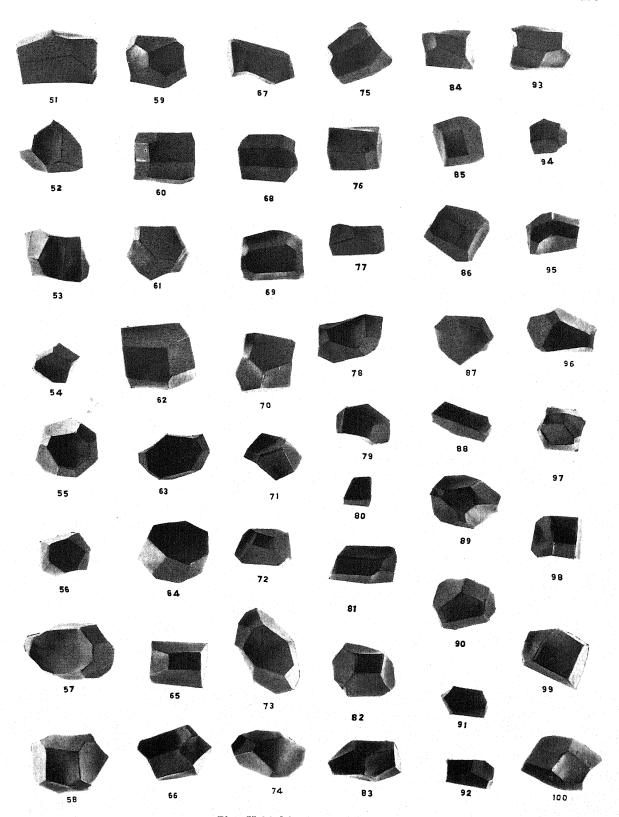


Plate II. Models of cells 51-100. ×85.

10 ac	Surface rhor dodecahedro	23.47	22.37	24.59	28.33	28.75	22.09	26.81	31.67	20.00	30.43	05.71	34.17	32.09	£0.1%	25.01	19.03	29.58	21.67	15.83	22.37	23.06	26.81	21.39	34.17	22.77	32.50	23.47	23.47	18.00	21.59	25.00	17.08	68 96	86.06	18.75	00 0%	08 90	96.30	10.70	94.17	14.44	14.44	10.00	10.01	17.50	20.09	
-ε	Surface orth tetrakaidece hedron of ec	23.34	22.24	24.45	28.18	28.60	21.97	99.96	31,50	19.89	30.26	12.71	33,98	51.91	24.1%	75.4.7 70.0.7	18.92	29.42	21.55	15.75	22.24	22.94	99.96	31.28	33.98	55.65	32.32	23.34	23.34	17.90	21.20	22.34 00.04	16.09	06.05	20.02	18.65	98.00	06 0A.	20.0% 36.10	10.64	#0.01	00'00 14.96	14,00	15,41	10.72	12.71	20.05	
	Surface of a sphere of a volume	21.24	20.24	22.25	25.64	50.95	19.99	24.26	28.66	18.10	27.53	15.71	30.92	29.04	20.02	22.03	17.32	26.77	19.61	14.33	20.24	20.87	24.26	19.36	30.92	20.61	29.41	21.24	21.24	16.34	19.30	10.0%	15.46	88.80	18.95	16.07	16.01	28.00	20.00 20.03	08.00	9.00	30.9% 12.07	10.01	14.08	15.21	10.01 080	23.23	
Э	Total volum cu. cm.	9.30	8.55	9.90	12.35	12.45	8.35	11.30	14.40	7.30	13.70	5.90	16.30	14.85	11.80	10.20	6.80	13.15	8.25	5.10	8.55	8.95	11.30	7.90	16.30	8.80	15.05	9.30	9.20	6.50	06.7	0.00	05.6	11.00	7.50	02.9	7.70	10.05	02.01	9.00	00.00	10.55	4.00	5.00	00.0	0.30	00.01	
	Total area sq. cm.	25.32	22.32	27.03	90.82	30.57	22.19	27.99	33.15	21.48	31.02	19.41	87.22	33,35	29.09	25.09	19.41	29.35	23.99	16.96	96.22	24.83	28.25	22.38	35.41	23.61	32.38	25.35	25.74	20.25	21.28	20.03	18.06	00.01	00.83	17.0%	11.55	00.1% 00.00	20.00	14.06	14.90	55,33 1.4.99	14.00	17.10	16.90	17.87	20.00	
	Total area sq. in.	3.91	3.46	4.19	4.35	4.74	3.44	4.34	5.14	3.33	4.81	3.01	5.77	5.17	4.51	3.89	3.01	4.55	3.73	2.63	3.56	3.85	4.38	3.47	5.49	3.66	5.02	3.93	3.99	3.14	3.30	20.00	00.0	70°°	00.0	03.0	2.03	10.0 10.0	4.00	9.00	25 T	20 C	02,2	2.66	20.00	:	4.04	
səa	Total no. fac	13	13	91	15	15	13	14	15	12	91	10	₩ ₩	15	91	14	12	15	Π	11	Π	13	Π	<u>2</u>	18	13	13	15	13	07	<u>n</u> ,	<u> </u>	CT CT	2 -	7 7	# C	3 5	2 5	2 2	0 5	2 ;	7. F.	T	3ì.;	11	Ξ,	2	
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ges per face and their areas	of 6-sided sees	2.55	45.	2.73	3.30	2.21	1.08	3.08	2.91	1.39	1.63	.59	1.25	2.34	1.88	1.26	1.84	47	1.10	1.18	2,38	1.59	1.74	2.08	49	2.14	2.47	9.61	.97	:	1.08	1.00	1.16	4. 5.	60.	1.84	89.		1.24	25	•	1.68	18.	1.51	1.15	1.05	1.60	
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Number of	sərs lətəT bəbiz-č to zəsst	.74	1.16	.93	.50	1,31	1.33	1.25	1.87	1.61	1.64	1.85	2.24	1.47	1.08	1.02	44	$\tilde{c}6.\tilde{c}$	2.07	76.	:	1.94	1.78	.64	5.60	98.	1.56	1.01	1.48	2.84	1.13	2.59	22.	7.87	42.7	1.25	08.	1.54	27.2	.75	7.	1.20	.97	.78	.85	1.11	2.05	
Nun	No. of 5- sided faces	c)	₹	4	CS.	10	4	က	9	9	9	9	9	5	7	4	C)	10	9	4	:	9	4	(0)		4	4	4	č	œ	9	00	ر د	0	٥	9	<b>20</b>	9	9	က	တ		4	4	₫	4	9	
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	of 3-sided sees	•	:		•	•				:	•	:	:	:	:	:	•	:				:	:		•			:	:	:	:	:	•	:	:	•		:	. ,	20.	<u></u> 60.	· •	•	:	:	:	٠:	
	No. of 3- sided faces Total area		:	•	•	•	:	•	•	:	•	:	:	:	:	:	:	:	:			:	:		:			:	•	:	•	:	•	•	•	:	:	•	• 1		,—	:	:	:	:	:	:	
	Cell no.	ī	C\$	က	4	10	9	2	<b>%</b>	6	10	=	13	13	7	Iş	16	17	18	10	06	16	00	60	76	25	98	22	86	67	30	31	ကို	SS 1	4, 6	35	36	37	88	36	40	41	<i>6</i> 45	43	44	45	46	

cu. cm., etc. From this it is evident that increase in the number of faces and increase in volume tend to go hand in hand.

Table 3. Frequency of occurrence of each kind of face and a ratio expressing their proportionate occurrence.

Number of edges							
per face	3	4	5	6	7	8	9
Frequency	4	418	471	363	71	7	2
Ratio	.04	4.18	4.71	3.63	.71	.07	.02
Approximate ratio	0	4	5	4	1	0	0

In table 3 the frequency of occurrence of the kinds of faces on the cells studied, a ratio expressing their proportionate occurrence, and also an approximate ratio to the nearest whole number are given. Pentagonal faces occur most frequently with quadrilaterals and hexagons next most common. On the basis of these frequencies, an hypothetical average cell would have 5 pentagonal faces, 4 each of quadrilateral and hexagonal faces, and 1 heptagonal face. No such cell occurred among the 100 studied, although there is no a priori reason why it might not be found.

The frequency of occurrence of each kind of face is given again in table 4, and in addition, the total surface area of each kind of face as measured on the models and the average surface area as derived from these totals. The average areas in square inches were converted to square centimeters, and from these data the average surface area of each kind of face occurring on the cells themselves was calculated. It is apparent that there is a direct correlation between the number of edges on a face and the

size of the face-that is, that the larger faces have more edges than the small ones. Thus, triangular faces have the smallest average area, and those with the greatest average area have the most edgesnamely, 9. The last column of table 4 shows the comparable areas of similar regular polygons. The length of a side for each of these regular figures is 1, since the regular hexagon, whose area was arbitrarily taken as 2.6 units, has unity for the length of each side. A factor for each of the regular figuresfor example, triangle, 0.4; square, 1.0; pentagon, 1.7; etc.—multiplied by the square of the length of a side gives the areas as recorded in the table. These areas and those of the corresponding cell faces are strikingly similar. Such relationships and their significance in epithelial tissue have been discussed by Lewis (1931).

A summary of the total and average number of faces on the cells, the total surface areas and volumes of the models and of the cells, and the surface areas of a sphere, an orthic tetrakaidecahedron, and a rhombic dodecahedron with volumes equal to the volume of the average model and of the average cell are given in table 5. The total number of faces on the 100 cells studied is 1,336 with an average of 13.36 faces per cell. The 100 pith cells studied by Lewis averaged 13.96 contacts. The total surface area of the cell models studied here is 2,465.35 sq. cm. Thus, an average model would have a surface area of 24.65 sq. cm., and by computation, since the models were drawn at a magnification of 170 diameters, an average cell would have a surface area of 85,294.12 sq. microns. It is interesting to note that this value (85,294.12) is not markedly different from the surface area of an orthic tetrakaidecahedron (82,006.92) or rhombic dodecahedron (82,525.96) of equal volume. The sum of the vol-

Table 4. The total numbers of different kinds of faces on 100 cells, their total and average areas as measured on the models in square inches and computed in square centimeters, and the computed average areas in square microns of the different kinds of faces on the cells themselves, and areas of comparable regular polygons.

	Total number	Total area sq. in. models	Average area sq. in. models	Average area sq. cm. models	Average area sq. microns cells	Area com- parable regular polygons
Number of 3-sided faces  Area of 3-sided faces			09	10	0.FW 4.4	0.4
		.11	.03	.19	657.44	0.4
Number of 4-sided faces  Area of 4-sided faces		60.83	.15	.97	3,356.40	1.0
Number of 5-sided faces Area of 5-sided faces		134.56	.29	1.87	6,470,59	1.7
Number of 6-sided faces					7,7,7,7	
Area of 6-sided faces		144.95	.40	2.58	8,927.34	2.6
Number of 7-sided faces Area of 7-sided faces		35.27	.50	3,22	11,141.87	3.6
Number of 8-sided faces Area of 8-sided faces		4.52	.65	4.19	14,498,27	4.8
Number of 9-sided faces		1.99	.99	6.39	22,110.73	6.2

Table 5. Summary of the total areas and volumes.

		Total	Average
Number of faces		1,336	13.36
Area of the faces on the models in sq. in		382.23	3.82
Area of the faces on the models in sq. cm		2,465.35	24.65
Area of the faces on the average cell in sq. microns	85,294.12		
Volume of the models in cu. cm		972.85	9.73
Volume of an average cell in cu. microns	1,980,460.00		
Surface areas of spheres with volumes equal to the			
volumes of the individual models in sq. cm		2,156.85	21.57
Surface area of a sphere with a volume equal to the			
volume of the average cell in sq. microns	74,636.68		
Surface areas of orthic tetrakaidecahedra with volumes			
equal to the volumes of the individual models in			
sq. cm		2,370.39	23.70
Surface area of an orthic tetrakaidecahedron with a	* .		
volume equal to the volume of the average cell in			
sq. microns	82,006.92		
Surface areas of rhombic dodecahedra with volumes			
equal to the volumes of the individual models in			
sq. cm		2,383.37	23.83
Surface area of a rhombic dodecahedron with a volume			
equal to the volume of the average cell in sq. microns	82,525.96		

umes of the models is 972.85 cc. with the average at 9.73 cc. By computation, the average cell would thus have a volume of 1,980,460 cu. microns.

The number of trihedral and tetrahedral angles was recorded: there are 6,732 trihedral angles and 14 tetrahedral angles on the 100 cells, or approximately 1 tetrahedral angle to 481 trihedral angles. In the cells, tetrahedral angles are much less common than in compressed lead shot of uniform diameter in which the proportion of tetrahedral to trihedral angles was 1 to 93. Matzke (1939) studied compressed lead shot of two different diameters mixed in varying proportions, and he found a ratio of 1 tetrahedral angle to every 251 trihedral angles, a result much closer to that found in cells than was obtained for compressed lead shot of uniform diameter. In addition, he found a correlation between the number of tetrahedral angles and the size of the faces in shot: small faces had more tetrahedral angles than large ones. This would be expected if the number of tetrahedral angles were determined merely by space relationships. The faces on the cells are relatively minute, and the fact that tetrahedral angles are much less common here than in compressed lead shot seems to indicate that surface tension has an influence on the position of the cell walls with reference to one another.

Plates III and IV are drawings of models, each of which is shown in three different views, selected to illustrate some of the variations in the shape, size, and arrangement of the faces. Cell number 20 (plate III, figures 20 a, b, and c, and table 1, number 20) is of interest not only because of the arrangement of the faces, but also because it is the only cell observed on which there are no pentagonal faces. It has a total of eleven faces, six quadrilateral and five hexagonal. The quadrilaterals are smaller than the hexagons and have a combined area almost

precisely half that of the latter. The faces are symmetrically arranged. The top, base, and three of the sides are hexagons. Alternating with these three sides are three others each of which has two quadrilateral faces. On two of these last-named sides one of the quadrilaterals is longer than the other, while they are approximately equal in size on the third side. Professor Lewis has suggested that cell number 20 may be derived from the orthic tetrakaidecahedron as follows: "the vertical division of two neighboring cells in contact with two adjacent lateral hexagonal facets converts the 14-hedron into a 16-hedron with heptagonal facets above and below. A vertical division through these heptagonal facets (somewhat unequal, but such as often occurs) produces a daughter cell which is hexagonal on its top and base (the other daughter cell is pentagonal above and below). A horizontal division through the middle of the hexagonal daughter cell gives rise to the pattern in question (in one of its halves)."

Cell 81 (plate IV, fig. 81a, b, c) has thirteen faces of which two are quadrilateral, eight pentagonal, and three hexagonal, thus showing to a marked degree the tendency in the group as a whole toward a predominance of pentagonal faces. The average for the 100 cells shows more quadrilateral than hexagonal faces, while in this case, the reverse is true. As in cell 20, the quadrilateral faces have the smallest average size. The pentagons are next largest (there were no 5-sided faces on cell 20) and, again as in number 20, the hexagonal faces are the largest. In this same cell the top and base are hexagons (81a, b), three of the sides have two pentagonal faces each (81b), two have both a quadrilateral and a pentagonal face (81a, c), and one side is a hexagonal face which appears at the top in figure 81c. In this one cell there occurs in succession a series of faces which show a progressive size increase, espe-

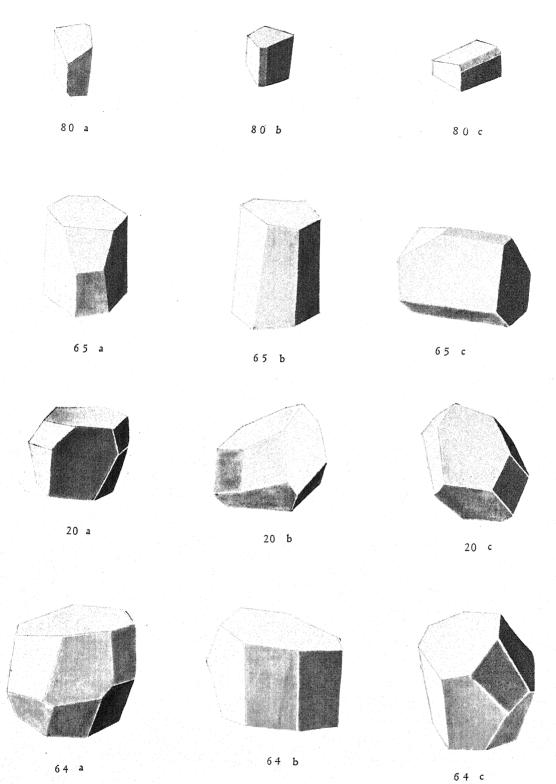


Plate III. Drawings of four different cell models, each in three views, numbered to correspond with their photograps in plates I and II.  $\times 170$ .

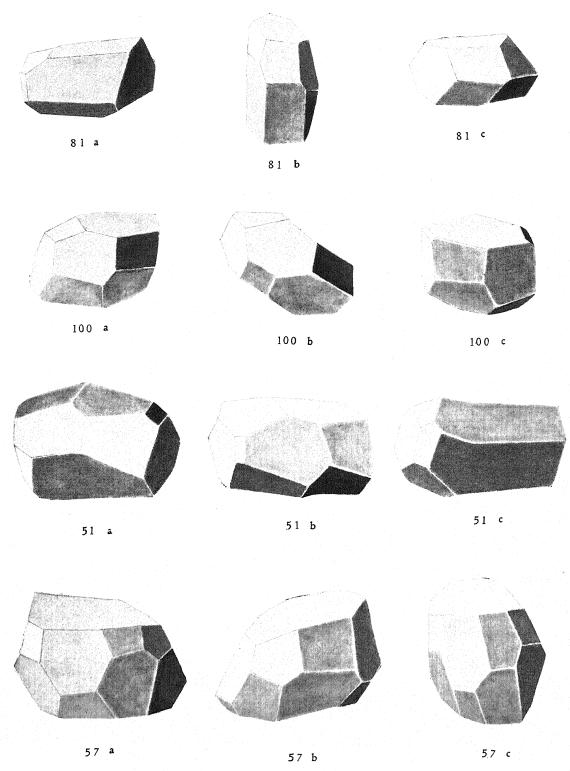


Plate IV. Drawings of four cell models, each in three views, numbered to correspond with their photographs in plates I and II.  $\times 170$ .

cially in proportion to the length of the side of the cell which they occupy. There are no tetrahedral angles on the cell, but trihedral angles are formed between each group of three faces. Cell 81 may be interpreted as a vertically bisected orthic tetrakaidecahedron; two adjacent cells also divided vertically, thereby introducing two additional facets to give cell 81 a total of thirteen contacts.

Cell 100, with 15 faces, of all the cells studied, most closely approximates the orthic tetrakaidecahedron. It has eight hexagonal, five quadrilateral, and two pentagonal faces. Two of the hexagons form the top and the base (only one of these is visible in the drawings, and it appears at the extreme left in figures 100a, b, and c); three adjacent sides (two of these visible in 100b) each have a quadrilateral and a hexagonal face in such a position that the quadrilaterals and hexagons alternate; a fourth side, in addition to a hexagonal and a quadrilateral face, has a small quadrilateral face (upper left-hand corner, 100c); the remaining two sides, each of which is adjacent to the side having the small quadrilateral, have a hexagonal and a pentagonal face apiece. If the small quadrilateral face were not present, the two pentagonal faces would automatically become quadrilaterals, and the number and arrangement of the quadrilaterals and hexagons would be identical with that of Lord Kelvin's figure. Except for this small face, then, this cell approaches the tetrakaidecahedron very closely, although even with the omission of the small quadrilateral it still would not be perfectly regular. The

Table 6. Frequency of occurrence of different combinations of faces which occur more than once on the 100 cells.

	N	umber of e	dges per f	ace
Number of cells	4	5	6	7
2	2	8	. 2	
2	3	6	5	
2	4	4	5	
2	4	5	5	1
2	4	6	2	2
2	4	6	3	2
2	5	2	5	٠.
2	5	4	1	. 9
2	5	4	4	ç
2	5	4	5	9
2	6	2	1	9
2	6	2	4	1 1 9
3	2	8		
3	3	6	1	
3	3	6	2	
3	3	6	3	· .
3	3	6	4	
3	4	4	4	
3	4	4	6	
3	4	4	7	
3	4	6	3	
3,	5	2	6	
3	5	2	8	
6	4	4	3	

quadrilaterals on this cell have a smaller average surface than the pentagons, which, in turn, have less surface than the hexagonal faces.

These three cells (20, 81, 100) illustrate the correlation between the size of the faces and the number of edges on a face. They show some of the facesize variations, and they also demonstrate the fact that when two faces occur on one side of a cell, one of them is usually smaller than the other, thereby placing the common edge to one side of the center of the cell. It can also be seen that, while patterns do occur, they are subject to considerable variation.

An analysis of the occurrence of the different kinds of faces found on the cells shows that in many cases two or more cells with an equal number of sides have not only the same kinds of faces but also like numbers of each kind. All of these were collected in table 6 where the combination of number and kind of face as well as the frequency of occurrence of these combinations is recorded. The faces are quadrilateral, pentagonal, and hexagonal, with a few heptagonal. Thus, there are two cells having two quadrilateral, eight pentagonal, and two hexagonal faces; there are two cells having three quadrilateral. six pentagonal, and five hexagonal faces, etc. There are twelve cases in which the same combination of faces occurs twice, eleven where it occurs three times, and in one case the same combination occurs six times. It is evident from the table that since there are twenty-four different combinations, no one can be said to be predominant, although it is rather striking that there are six cells with four quadrilateral, four pentagonal, and three hexagonal faces. A bisected orthic tetrakaidecahedron also has four quadrilateral, four pentagonal, and three hexagonal faces. The arrangements of these faces in the cells and in the bisected models, however, are not the same, and variation occurs between the individual

A further analysis of table 6 indicates that pentagonal faces predominate when the number of quadrilaterals and hexagons is small, and conversely, that when the quadrilateral and hexagonal faces are present in greater numbers, there are fewer pentagons.

Discussion and conclusions.—The 100 cells of Eupatorium purpureum L. pith isolated and studied have an average of 13.36 contacts. This average approaches an average (13.97) obtained by Lewis (1923, 1925, 1933) in a study of 100 elder pith cells, 100 human fat cells, and 50 precartilage cells of the tadpole, all essentially undifferentiated tissues. The range in the number of contacts, 7–20, observed in Eupatorium pith is almost identical with the range obtained by Lewis for 100 elder pith cells.

Studies of the effect of contact and pressure on lead spheres have demonstrated that while spheres of uniform diameter have an average of 14 (14.17) contacts when compressed to eliminate all the spaces, spheres of two different diameters, one twice the other, when mixed together and compressed give averages in which the small shot always have fewer than 14 contacts and the large shot more than 14.

In this same series of experiments, varying the proportion of small shot to large changed the average number of contacts for each group. Thus the small shot averaged 9.5 contacts when there were relatively fewer small shot in the mixture, and the average rose to 13.3 contacts when the proportion was relatively high. The average for the large shot was 19.98 faces when there were more large than small shot by volume and rose to an average of 30.18 contacts when there were more small than large shot by volume. The cells, divided into two groups according to volume, show averages of 12.78 contacts for the small cells and 15.41 for the large ones. As in the lead shot, the small cells have fewer than 14 contacts, while the large ones have more than 14. The marked differences observed in shot would not be expected here since the diameter ratio of small cells to large ones (computed on a basis of spheres for comparison) was 1 to 1.26, considerably less than the diameter ratio of 1 to 2 used in the lead shot study, and the mean diameters of the cell models, 2.48 cm. and 3.12 cm., are based in each case on a considerable range in the diameters of the individuals. However, the correlation between cell size and the average number of faces in a population of cells of different sizes, on the one hand, and the effect of contact and pressure on spheres of two diameters, on the other, is apparent.

Harper (1918a, 1918b), in a consideration of the morphogenetic factors involved in shape determination in certain algae, discussed the effect of contact and pressure on cells whose primary form was determined by surface tension. In the lead shot, surface tension is reduced to a minimum, and distortion from a spherical form is induced by external pressure. In pith the polyhedral cells do not normally develop from spheres; nevertheless the similarity to forms produced by distortion is striking. Harper (1918b) has also emphasized functional hypertrophy as a stimulus in cell form determination, and Priestley (1928) is of the opinion that internal pressure is the force responsible for the polyhedral form of meristematic cells.

The Eupatorium pith cells frequently appear oriented in rows parallel to the long axis of the stem. They are often elongated, and since divisions are commonly transverse, cell division does not change the number of rows of cells. In longitudinal section they appear as in figure 2. Certain of these cells may be looked upon as short or elongated modified prisms each with a single face at top and bottom and with 4 to 8 sides any one of which may consist of more than a single face (fig. 1, 2). Plates III and IV show drawings of eight cell models, each in three views, which have been selected to show several modifications of an essentially prismatic form.

Figures 80a, b, and c (plate III) give three different views of cell number 80, which is an irregular pentagonal prism. There are two pentagonal faces which form the top and base and five quadrilateral faces forming the sides of the figure. This cell might arise from the transverse division of a vertically bisected orthic tetrakaidecahedron if the plane of the

transverse division were one-third of the distance from top to base. Figures 65a, b, and c show that cell 65 is hexagonal in cross-section, has three quadrilateral and two pentagonal faces extending from the top to the base, and a sixth side consisting of two quadrilateral faces, each of which was a contact of an adjacent cell. Cell 20 also has a hexagonal face at the top and base, three lateral hexagonal faces extending from top to bottom, and three other sides, each divided into two quadrilaterals. Thus each hexagonal face alternates with two quadrilateral faces. In cell 64 (fig. 64b and c) there are two quadrilateral and two pentagonal faces extending the length of the cell. The other three sides (fig. 64a) are each divided into two faces. These lateral faces may be narrow and extend a short way along the cell, or they may extend to a point more nearly equidistant from the top and base. Such variations are shown in cell 81 (plate IV, fig. 81a, b, c). Not uncommonly three lateral faces occur on one side of a cell, and Lewis (1923) has pointed out that "this happens whenever, in an aggregation of orthic tetrakaidecahedra, a cell adjacent to a lateral hexagonal facet divides transversely through the middle." In such an event one or sometimes two of these faces are rather small (plate IV, fig. 51a, 100c). Cell 100, previously described, approaches the orthic tetrakaidecahedron in shape. Figures 100a and b show the necessary alternation of quadrilaterals and hexagons. On figure 100c, however, a narrow quadrilateral face appearing at the upper left increases the number of faces to fifteen and changes two of the quadrilaterals to pentagons, thus destroying the orthic tetrakaidecahedral pattern. The greatest number of faces observed occur on cell 57 which has 20. Its model with a volume of 22.3 cc. is within 0.2 cc. of the volume of the largest cell studied, number 73, which is 15-sided.

From plates III and IV it is evident that the size and position of the faces are subject to great variation. The delicate curvatures which some of the faces originally had could not be modeled. In surface tension systems the curvatures are important from the standpoint of stability of angles. Only rarely are four adjacent faces so arranged that a tetrahedral angle is formed. In all the 100 cells there are only 14 tetrahedral angles, while trihedral angles number 6,732. Two of these tetrahedral angles are shown in plate II, figures 60 and 84, and two others in plate I, figures 6 and 30. As has already been pointed out, these angles are much less frequent here than on compressed lead shot of uniform or of different sizes in which surface tension probably plays little or no part. In cells, the operation of surface tension and biological factors would reduce the occurrence of this type of angle. As seen in section, the cells "break joints," and from the models it is apparent that two cells forming one side of an adjacent cell usually have their end walls toward one end rather than in the middle of that adjacent cell. It has already been mentioned in connection with cell 81 (plate IV) that this position varies.

In discussing table 3 it was pointed out that pentagonal faces occurred most frequently with quadrilateral and hexagonal faces next. This is also apparent from plates I, II, III, IV, and table 1. This predominance of pentagonal faces was observed in the studies of compressed lead shot of uniform and of different diameters under most conditions. In the studies on shot of different diameters, the small shot showed more pentagonal faces than any other kind. Lewis (1923, 1925) has pointed out that in elder pith two pentagonal faces frequently occur together. He suggested that this arrangement may represent a modification of the regular alternation of quadrilateral and hexagonal faces found on the orthic tetrakaidecahedron and that greater economy of surface may thus be obtained.

A study of cells of equal volume shows that while cells of the same volume may have the same number of faces, they frequently do not. The considerable range in cell volumes evident from table 1 has already been discussed in the light of its effect on the number of faces; small cells usually have fewer faces than large ones. The surface areas of the cells are also affected by their volumes: small cells have less surface than large ones. A comparison of individuals, both large and small, with regular polyhedra of equal volume shows that the small cells frequently are as economical surface-volume figures as the large cells, and in some cases they are more economical. There are seven small cells (table 1, nos. 36, 59, 71, 72, 86, 87, 90) which are more economical and three (table 1, nos. 30, 56, 80) which are as economical surface-volume figures as orthic tetrakaidecahedra of equal volume. In addition, there are four other small cells which show more economy of surface than rhombic dodecahedra of the same volume. Of the large cells, four (table 1, nos. 4, 17, 39, 58) are more economical than orthic tetrakaidecahedra and two others (table 1, nos. 26, 41) are more economical than rhombic dodecahedra of equal volume. The other 80 cells in the 100 studied have surface areas that are greater than those of either an orthic tetrakaidecahedron or a rhombic dodecahedron of equal volume. However, the differences are often small and possibly no greater than the experimental error. Too much weight, therefore, should not be put on the individual figures. But it may be concluded that both large and small cells can be as economical surface-volume figures as orthic tetrakaidecahedra of equal volume and that in this study no direct generalization between large and small cells can be made in this respect. The striking result of the volume studies is that the cells approach the economical surface-volume relationships of the regular figure so closely. This would indicate that surface tension is important in cell shape determination.

In spite of the differences in materials and methods, the average number of contacts observed for the *Eupatorium* pith cells is rather close to that obtained by Lewis for 100 elder pith cells. Pentagonal faces were most common in the elder pith and are

the most common kind of face (471 in all) observed on the cells studied here. However, quadrilaterals and hexagons are also numerous (418 of the former and 363 of the latter), and the heptagons number 71. In contrast there are only 7 octagons, 4 triangles, and 2 faces with nine edges. While no one kind of face is strongly predominant, the majority definitely occur as three types, faces with 4, 5, and 6 edges, with a fourth type, 7-edged, less frequent.

An examination of the photographs of the cell models (plate I, II) does not reveal a uniformity of pattern, although their shapes may often be interpreted as a modification of the orthic tetrakaidecahedron as shown above. In addition to differences in shape, variation in the kind and number of each kind of face is evident. A study of table 1, however, shows that there are cells which have not only the same kinds of faces but also precisely the same number of faces and of each kind of face-for example, cells 50 and 77 both are 10-sided with two quadrilateral and eight pentagonal faces. In the other twentythree cases (table 6) in which the cells have the same numbers of faces of the same kind, the arrangement of the faces may or may not be similar. It is interesting to note that in twelve of these twentyfour cases similar combinations appear only twice, in eleven cases three times, and in one case six times. This last case comprises 11-sided cells with four quadrilateral, four pentagonal, and three hexagonal faces, all polygons of frequent occurrence. The fact that twenty-four different combinations occur in the 100 cells seems to preclude the possibility that any one pattern is representative of the cells as a group. Although the orthic tetrakaidecahedron may well occur in cells, such a typical figure was not observed here, and these data indicate that it does not occur more frequently than other combinations of faces.

Cell division and cell expansion among the biological factors have a marked effect on cell shape determination. However, some aspects of shape determination can be studied in systems in which these factors are not operative. Thus, lead spheres of equal volume compressed to eliminate interstices averaged 14 faces of which pentagons were the most numerous. As previously noted, the effect of size differences on the shape of compressed lead spheres has also been demonstrated to affect the number of faces markedly. The cell data presented here give similar results and emphasize the importance of contact and pressure in cell shape determination.

The assumption that semi-liquid, colloidal, and plastic cells tend within limits to assume a least-surface to volume configuration is substantiated by a comparison of the surface areas of the cells with the surface areas of orthic tetrakaidecahedra of equal volume. Some of the cells observed have surface areas as economical as those of orthic tetrakaidecahedra of equal volume, and most of the cells approach the economy of surface found in this regular figure.

A study of the 100 pith cells modeled indicates that the number of faces varies most commonly between eleven and fifteen, with an average of 13.36. The cells studied may be divided into two groups, the larger ones with an average of 15.41 faces, the smaller ones with an average of 12.78, and the smaller ones are considerably more abundant than the larger. If the theoretical average for cells of uniform size is 14, as previous studies would indicate, then the preponderance of smaller cells would tend to reduce the average below 14 in the present study. Among the 100 cells there is no predominant type or pattern that is clearly evident. Six cells had four quadrilateral, four pentagonal, and three hexagonal faces, and none of the other types occurred more than three times among the cells modeled. While the average clearly approaches fourteen, it is difficult on the basis of these data to resolve these cells into one ideal shape or pattern with a definite number of hexagonal, pentagonal, and quadrilateral faces, although certain of the cells can easily be interpreted as modified orthic tetrakaidecahedra. Surface tension and contact and pressure are ostensibly of major importance in cell shape determination.

Any average or typical fourteen-sided cell would tend to become obliterated by the numerous modifying influences, such as variations in size, division of certain cells in the tissue, changes in relative viscosity, or other factors, biological as well as physicochemical. Either there is no very definite pattern superimposed on the polyhedron which is fourteen-sided, or else it becomes obliterated; the result is the same—none could be found in the mature cells

modeled and studied.

#### SUMMARY

Three-dimensional models of 100 Eupatorium pith cells were made by means of a new technique previously described.

These cells had an average of 13.36 contacts, an average rather similar to that (13.97) found by Lewis for 250 undifferentiated cells. The average number of faces approaches the average for compressed lead spheres of uniform diameter (Marvin), but the averages for large and small cells tend to resemble those found by Matzke for large and small compressed lead spheres. Assemblages of polyhedra with trihedral angles only, yet averaging less than 14 faces, are possible through certain planes of division (Lewis).

The average cell, oriented in vertical columns, presents an upper and a lower facet, with 11 or 12 lateral facets. Pentagonal faces are most common

on the cells as on compressed lead shot.

There is a correlation between the size of a face and the number of its edges: large faces have more edges than small ones. The areas observed are similar to the areas of the corresponding regular polygons with sides of unit length.

The cells show an economy of surface to volume approaching, and in some cases equalling, that of an orthic tetrakaidecahedron or a rhombic dodeca-

hedron of equal volume.

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## A CASE OF CORRELATIVE GROWTH INHIBITION IN PLANTS <sup>1</sup>

F. W. Went

In the extensive work on substances affecting the growth rate of Avena coleoptile cells, practically all substances tested are either inactive or increase the growth rate. In only a few instances growth inhibitions have been reported. Almost all were demonstrated by applying a small agar block containing the substance in dissolved form to one side of the cut surface of a decapitated Avena coleoptile (Avena test). If the substance increases the growth rate, the plant will bend away from the side with the agar block (negative curvature); with a growth retarding substance a positive curvature should result. Gorter (1927) dismissed all previously recorded cases of growth inhibitors by demonstrating that in all those cases the positive curvature appeared more than 2½ hours after the last decapitation of the test plant. Positive curvatures, appearing after that period, can be accounted for by regeneration of auxin at the free cut surface, not covered by agar. Later Czaja (1934) and Laibach and Meyer (1935) reported positive curvatures with certain plant extracts, Czaja attributing them to tannins. For a more complete survey of the literature see Went and Thimann (1937). Since then a real growth inhibitor has been found and extracted (Stewart et al., 1939). The following report, however, is concerned only with substances inactive in or promoting growth.

The case of lateral bud inhibition is, of course, the best known correlative growth inhibition known. In a recent paper the author (Went, 1939a) discusses this case in the light of experiments similar to those presented in this paper, so that no further mention will be made of bud inhibition. In another paper it is shown (Went, 1939b) that in the pea test for auxin a substance must possess at least two different properties to cause the peculiar growth curvatures in the split pea stems—namely, to induce the preparatory and the growth reaction. Some substances, like indole acetic acid, are highly effective in both properties; others, although able to produce the preparatory reaction, may have diminished growth properties (phenyl acetic acid) or may have no growth promoting effect at all (hemi-auxins like cyclohexane acetic acid and γ-phenyl butyric acid). As will be seen, a clear distinction between these three groups of substances is advantageous for the understanding of the following facts.

THE FACTS ABOUT THE GROWTH INHIBITION IN AVENA COLEOPTILES BY GROWTH PROMOTING SUBSTANCES.—A. Indole acetic acid.—When high concentrations of indole acetic acid (e.g., 20 mg./l.) in agar are unilaterally applied to decapitated Avena coleoptiles, negative curvatures develop within one hour, continuing for many more hours. Occasionally there will be found a coleoptile, which shows a positive curvature below the negative. A picture of such

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a positive curvature can be found in Went and Thimann (1937), fig. 22, the extreme right-hand plant of the lowest row. Although the general rule is that indole acetic acid causes growth acceleration, in certain rather exceptional cases (using high concentrations) it thus may produce a growth inhibition near the base of the coleoptile.

The same phenomenon may be observed with direct growth measurements. If the growth of the zones of an Avena coleoptile be measured, a distinct distribution of the growth rate will be found (see e.g., Went, 1928, 1935). Upon application of a high auxin concentration in lanolin to the tip of the coleoptile, in all cases the growth of the middle zones will be greatly increased (see fig. 2), but again in a few individual plants the growth rates of the lowest growing zones may be decreased. Thus, although in each coleoptile the gross growth effect is an acceleration, indole acetic acid may cause a local growth inhibition of the basal zones. Isolated basal sections invariably show a growth acceleration when immersed in an indole acetic acid solution.

Because the phenomenon is too uncommon when indole acetic acid is applied it will be studied in greater detail with other compounds, having a smaller or no growth effect.

B. Phenyl acetic acid.—High concentrations of phenyl acetic acid (30 mg./l. or more) in agar, when unilaterally applied to the cut surface of decapitated Avena coleoptiles, will induce distinct negative curvatures in the extreme tip of the plants only. But below this negatively curved zone a much larger positive curvature develops from one and one-half to three hours after putting the agar on (see fig. 1A). Here the apical and basal zones simultaneously bend in opposite directions. Thus in this case, where the growth acceleration is much less than with indole acetic acid, the growth inhibition of the lower zones is more pronounced.

If not only the curvatures are considered, but also the actual growth response of the zones of the coleoptile, when lanoline paste containing phenyl acetic acid is applied to the tip of the plant, the growth of the highest zones is accelerated, but more than

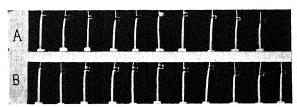


Fig. 1. Shadow pictures of Avena coleoptiles taken 165 minutes after applying agar.—Upper row (A): phenyl acetic acid 30 mg./l. applied in the agar block; S-shaped curvature (at top short negative curvature).—Lower row (B): cyclohexane acetic acid 20 mg./l. applied. Purely positive curvatures.

3 mm. below the tip the growth is greatly inhibited (see fig. 2). From another experiment can be drawn the conclusion that the higher the concentration in the lanoline, the greater the growth acceleration near the tip and the more extensive the growth inhibition in the base.

C. Cyclohexane acetic acid.—If a 20 mg./l. solution of cyclohexane acetic acid is applied unilaterally to the cut surface of an Avena coleoptile, no trace of negative curvature becomes visible, but within two hours distinct positive curvatures de-

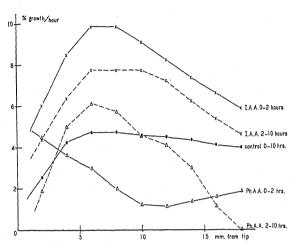


Fig. 2. Growth of the different zones of intact Avena coleoptiles (abscissa: distance of zones in mm. from tip), in percentage elongation of zone per hour (ordinate), when either pure lanoline was applied to the tip (control; black dots, growth distribution essentially identical from 0-2 and 2-10 hours after application of lanoline), or indole acetic acid paste (3 mg./g. lanoline; crosses, strong growth increase in all zones in both periods after application) or phenyl acetic acid paste (3 mg./g. lanoline; triangles). In the latter case a short apical zone of 4-10 mm showed an increase in growth rate over the controls, the lower zones a growth inhibition. The decrease in the top zone for the 2-10-hour period may have been due to slight injury. Each curve mean of 4 individual plants.

velop, which increase in size for a long time (see fig. 1B). Unlike the negative, these positive curvatures do not start in the extreme tip, but develop first at some distance from the cut surface. Substances like γ-phenyl butyric acid and indole-2-carbonic-3-propionic acid, also completely lacking any growth properties (see Koepfli, Thimann, and Went, 1938), behave in the same way.

Thus the substances mentioned under A, B, and C all are able to induce growth inhibitions in Avena coleoptiles, especially near the base; this effect may be partially (phenyl acetic acid) or almost completely (indole acetic acid) overshadowed by the growth acceleration.

D. Direct growth measurements with different substances.—A number of experiments were performed with Avena coleoptiles, on whose tip a dab of lanoline paste was stuck. Usually growth was

Table 1. Increase in length of intact Avena coleoptiles on which a dab of paste was put on tip. Mean of 6 plants each.

			th in n. in	
	Concentration		Next 13 hrs.	
Indole acetic acid	0.4 mg./g. lanoline	10.5	10.8	
Phenyl acetic acid	4 mg./g. lanoline	5.8	2.7	
$\gamma$ -Phenyl butyric acid.	4 mg./g. lanoline	2.2	1.8	
Pure lanoline		5.7	7.7	

measured twice in those coleoptiles: after 5 hours and after 18 hours. Table 1, which represents a typical experiment, shows that the indole acetic acid did increase the growth over the pure lanoline controls in both periods. In the first period the growth effect of the phenyl acetic acid kept the inhibition in check, but in the second period it gave a strong inhibition. And the  $\gamma$ -phenyl butyric acid only caused a growth inhibition, in both periods.

Here the question could be raised, whether the effect of the applied substances was on the auxin production by the tip. Therefore paste was applied to the tips of intact coleoptiles, and three hours later these tips were placed on agar. It was found that with phenyl butyric acid 2 mg./g. lanoline exactly the same amount of auxin was produced as with pure lanoline. With phenyl acetic acid 2 mg./g. about 40 per cent less auxin was collected. We must conclude then that the growth inhibition is not in the first place due to a lack of auxin. In a number of other experiments it was found that some substances, like indole carboxylic acid and the lowest concentration of y-phenyl butyric acid, did not cause any change in the rate of growth of intact or decapitated coleoptiles, as compared with the pure lanoline controls. Others, like indole acetic, indole butyric, and anthracene acetic acids, caused a strong increase in growth rate in both periods, whereas indole propionic and cis-cinnamic acids were much less effective. But again, y-phenyl butyric acid inhibited growth, in the highest concentration at least. Other experiments gave similar results.

Thus, the conclusion is well established that the substances mentioned above inhibit growth, especially of the more basal zones, of intact or decapitated *Avena* coleoptiles.

MECHANISM OF THE OBSERVED GROWTH INHIBITION.—There are at least two ways in which we can envisage the action of a growth inhibiting substance. In the first place it is possible that the substance directly interferes with the growth process. This means that we have to consider that in the growth process, requiring n factors, another has been introduced, so that n+1 factors cause a decreased growth rate. A simpler explanation is found in the assumption that one of the n factors required for growth is eliminated by the action of the substance. But not

Table 2. Three 4.2 mm. sections cut from coleoptiles (each figure mean of 10 sections). For 18 hours these are submerged (on combs) in the solutions indicated. For the next 24 hours all sections are transferred to a solution of indole acetic acid 1 mg./l. For this second period only the mean of upper, middle, and lower sections are given.

			First 18	hrs. in s	olutions		sect	xt 24 hrs ions in i c acid 1	ndole
	Concentra-		Section		Mean of all	Net increase over	Mean of all	In- crease over	In- crease over
Compound		Upper	Middle	Lower	sec- tions	con- trols	sec- tions	first period	con- trol
Water control		46.0	45.2	45.4	45.5		48.4	2.9	
Indole acetic acid	1  mg./l.	52.3	49.9	47.0	49.7	4.2	51.3	1.6	2.9
Indole acetic acid	0.1  mg./l.	49.0	48.1	$47.3^{-}$	48.1	2.6	50.0	1.9	1.6
Phenyl acetic acid	100  mg./l.	50.3	48.5	47.5	48.8	3.3	51.5	2.7	3.1
Phenyl acetic acid	10  mg./l.	49.2	48.4	47.0	48.2	2.7	50.4	2.2	2.0
Phenyl acetic acid	1  mg./l.	45.5	45.4	45.6	45.5	0	47.4	1.9	1.0

until the different growth factors are known can the latter explanation be attempted. Thus only after the rôle of auxin in growth became known, could most cases of traumatotropism be transferred from the former to the latter explanation.

And in the underlying case, where just the effect of auxin-like substances is to be elucidated, auxin cannot be the growth factor which is eliminated. This is clearly indicated by the experiment on auxin production, described above. Besides, the results of growth measurements were essentially the same when decapitated—that is, auxin-deficient—coleoptiles were used as with intact coleoptiles (in the cases of sections A, B, and D this has been studied in detail). Finally, in most of the following experiments indole acetic acid was applied in excess, so that there was no limitation by auxin-like substances.

Another growth factor, about which something is known, is the "food factor." This factor comes from the base of the coleoptile, and some growth phenomena could be explained by the assumption that auxin affects its upward transport (Went, 1935). To eliminate this supposed effect, coleoptiles were cut into short sections, which were then submerged in the solutions to be tested. In these sections a certain amount of food factor was "trapped," and applied auxin could not affect its supply any more, since the region of supply (the seed) was absent.

In one experiment three successive sections of 4.2 mm. were cut from a series of coleoptiles, which were then treated with tap water, indole acetic acid, or phenyl acetic acid (see table 2). For details concerning the technique of these determinations see Schneider (1938). From the table it will be clear that a section from any portion of the coleoptile responded to phenyl acetic acid with growth in exactly the same way as they responded to auxin, the only difference being that the concentration of the former had to be about 100 times higher. Since all acids were always made up with the equivalent amount of NaOH, and then diluted with distilled

water, the effects are not due to different pH. In these sections, especially the lower, no trace of growth inhibition with phenyl acetic acid was found. If afterwards placed in a solution of indole acetic acid, all sections grew still more, those previously in phenyl acetic acid even more than those in indole acetic acid. This proves that the sections were in no way injured by the phenyl acetic acid, so that on coleoptile sections at least it acts as an ordinary growth promoting substance, without any indication of growth inhibiting properties.

That also substances which cause only growth inhibition in the intact coleoptile have no inherent inhibiting properties on cells as such can be seen from table 3, compiled from data Dr. J. van Over-

Table 3. Growth of 6.4 mm. sections of etiolated pea stems in percentage of initial length, 4 and 24 hours after they were placed in the solution. Data collected by Dr. J. van Overbeek.

		Growth in percentage original leng	
Compound	Concentra-	In 1st	In
	tion	4 hrs.	24 hrs.
Indole acetic acid Indole acetic acid Indole acetic acid	3 mg./l.	30.9	61.1
	0.3 mg./l.	31.6	52.4
	0.03 mg./l.	20.7	39.3
Phenyl acetic acid	143 mg./l.	23.2	59,3
Cyclohexane acetic acid	590 mg./l.	14.4	23.8
Cyclohexane acetic acid	59 mg./l.	13.4	25.0
Cyclohexane acetic acid	5.9 mg./l.	11.1	20.3
Water control		10.4	21.0

beek was kind enough to furnish. In this case sections from etiolated pea stems, about 10 mm. below the tip, were used. They were placed in the solutions indicated and measured after 4 and 24 hours. Indole acetic and phenyl acetic acid behaved in the same

Table 4. Growth in mm. of 4.20 mm. long sections of Avena coleoptiles in indole acetic acid I mg./l. cut immediately after a 2- or 3-hour treatment of the coleoptile tip with the compounds mentioned in the first column. Measured 24 hours after sectioning.

	Expe	eriment 1	, paste or	2 hrs.	Expe	riment 2,	paste on	3 hrs.
	Gro	wth of se	ections in	mm.	Gro	wth of se	ctions in	mm.
Coleoptile tip treated with lanoline containing:	Тор	Upper middle	Lower middle	Base	Тор		Lower middle	Base
3 mg. indole acetic acid/g	.93	1.00	.98	.87	.33	.31	.31	.22
3 mg. phenyl acetic acid/g	1.41	1.51	1.21	.67	.63	.59	.30	.18
3 mg. γ-phenyl butyric acid/g	1.39	1.38	.81	.73	.64	.43	.27	.20
Pure lanoline	1.36	1.09	1.08	.86	.44	.33	.34	.22

way as in table 2, that is both were true growth promoting substances. But the cyclohexane acetic acid, which induces positive curvatures in *Avena* (section C above), did not decrease the growth rate; if anything, a slight acceleration could be observed.

So far, the results obtained here seem in contradiction with those described earlier in this paper. The difference, however, lies in the plants, which were cut into short cylinders in the one and intact in the other case. This makes it clear that the inhibition is a correlation phenomenon, connected with the distribution of growth factors inside the growing organ, and most likely the factor affected is the food factor. To collect further evidence for the latter viewpoint, the following experiments were carried out:

Avena coleoptiles were treated with a dab of lanoline paste containing the active substances, which was stuck on the extreme tip of the coleoptile. After two or three hours the 2 mm. tip with paste was cut off and the rest of the coleoptile cut into four sections, each 4.2 mm. long, after removal of the primary leaf by pulling it out from the base. The growth of these sections in an auxin solution containing 1 mg. indole acetic acid per liter was measured after one day; the results of two experiments are summarized in table 4. If it is considered that in all cases auxin was in excess, it must be concluded that differences in growth were due to differences in other growth factors, that is especially in the "food factor." Since we know from the experiments from tables 2 and 3 that the substances used did not injure the sections, we come to the following results: pretreatment with y-phenyl butyric acid increased the growth of the upper sections but decreased the growth of the sections from the lower part of the coleoptile; pretreatment with phenyl acetic acid caused exactly the same effect. These results lead to the conclusion that apical administration of both phenyl acetic acid and y-phenyl butyric acid caused a redistribution of the food factor inside the coleoptile in such a way that part of the food factor originally present in the base was moved towards the more apical zones. With indole acetic acid this effect was in most cases obscured by its effect on growth during the 2 to 3 hours of pretreatment. Under the influence of the auxin the food factor was apparently used up in growth more rapidly than it was supplied (see e.g., Went, 1935), so that the final effect was a decrease in growth rather than an increase. That this explanation is correct is indicated by an experiment in which there was an increase (except in the topmost section) in growth of the sections of plants which were pretreated with indole acetic acid for one hour only.

Since these experiments were carried out, a paper by Schneider (1938) appeared in which he clearly showed that sucrose is at least one of the components of the food-factor complex. Therefore, the preceding experiments were repeated by placing the sections in a solution containing 1 mg./l. of indole acetic acid and 1-2 per cent sucrose. If the effect of the applied substances were on the sugar in the coleoptile, then this effect would disappear when placed in an excess of sucrose. But if some other component of the food factor complex were affected, the effect would remain or even be more clearly pronounced, since the sugar would not be limiting the growth. In table 5 the data of three experiments are summarized. The differences in growth of the sections after the different treatments are more pronounced than in the experiments of table 4, which indicates that to some extent sugar was limiting especially in the most rapidly growing regions, as might have been expected from the results of Schneider (1938). But it also becomes clear that something else besides sugar has been affected by the phenyl acetic and phenyl butyric acid. This clearly demonstrates that besides sugar there are other factors present in the food-factor complex. Therefore Schneider's criticism (1938), based on experiments with sugar, of the limiting factor scheme of growth as proposed by the author (see Went and Thimann, 1937) does not necessarily hold for the auxin-food factor interrelations inside the growing region.

In all experiments (of which only three are reproduced in table 5) a clear (and in most cases statistically highly significant) increase in the growth of the upper 3 or 4 mm. appeared, and in eight out of ten cases a simultaneous decrease in the growth of the basal section was found.

The same experiments were performed with peas, to find out whether the redistribution effect of growth rate is a more general phenomenon. Since the situation is complicated in peas by the growth

Table 5. Growth in percentage after 24 hours of 3.1 or 4.2 mm. Avena coleoptile sections, placed in 1-2 per cent sucrose + 1 mg./l. indole acetic acid. Two hours previous to cutting these sections, lanoline paste containing different substances was placed on the extreme tip of the coleoptile.

Experiment 1		3.1	mm. secti	ons cut fro	om
Substance	Concentration	Тор	Upper middle	Lower middle	Base
Plain lanoline		13±1.3	22	15	12±1.7
γ-Phenyl butyric acid	5  mg./g.	$28 \pm 1.3$	29	15	$6 \pm 1.2$
γ-Phenyl butyric acid	2  mg./g.	$23 \pm 1.2$	17	15	9
Phenyl acetic acid	5  mg./g.	15	23	20	$5 \pm 1.5$
Added to the medium		1.6% su	crose		
Experiment 2		4.2	mm. sect	ions cut fr	om
Substance	Concentration	Тор	Upper middle	Lower middle	Base
Plain lanoline		11±1.2	15	8	4
γ-Phenyl butyric acid	5  mg./g.	$17 \pm 1.7$	18	11	7
γ-Phenyl butyric acid	2  mg./g.	12	12	5	2
Phenyl acetic acid	$2 \mathrm{mg./g.}$	$17 \pm 1.4$	15	10	5
Added to the medium		1.0% su	crose		
Experiment 3		4.2	mm. sect	ions cut fr	rom
		Province of the Control of the Contr	Upper	Lower	
Substance	Concentration	Top	middle	middle	Base
Plain lanoline		25±1.9	23	18	$17\pm1.0$
γ-Phenyl butyric acid	5  mg./g.	$29 \pm 1.3$	28	16	9±0.6
Added to the medium		2.0% su			

inhibition in the apical zones accompanying the swellings which appear after application of higher auxin concentrations, no detailed discussion will follow:

In the pea stems also the growth of the basal zones is inhibited by the more apical ones, since, after cutting these zones into sections, they grow more than on the intact plant. The same phenomenon was more clearly apparent when indole acetic acid paste 2.5 mg./g. was applied. On plants attached to their seeds the zone from 27–34 mm. from the tip grew 1.5 per cent in 24 hours. When the plant was cut off above the cotyledons, this growth was  $3\pm0.4$  per cent, but when this section was cut out from the plant, it grew  $6\pm0.3$  per cent. Thus also here apically applied auxin causes an inhibition in the lower zones of the intact pea stems, although by itself, on the isolated tissue it increases its growth.

Another phenomenon, mentioned in an earlier paper (Schneider and Went, 1938), now finds a ready explanation in the redistribution of food factor. When two hours before decapitation a small dab of auxin lanoline paste is applied to the tip of an Avena coleoptile, then in the subsequent Avena test the response of these plants to unilateral auxin application will be different. In the first place the

curvature is limited to a shorter zone near the tip of the coleoptile, and secondly the radius of curvature is decreased. This experiment was repeated several times. When indole acetic acid paste was applied, the response was not as consistent as with ciscinnamic, phenyl acetic or phenyl butyric acids. Table 6 lists some of the curvatures obtained when a 0.2 mg./l. indole acetic acid solution was applied in agar blocks to one side of coleoptiles, which had been treated for two hours with various auxins and hemi-auxins. From the length of curved zone and the total curvature the mean curvature per mm. coleoptile has been calculated. It will be seen that not only the curved zone was shorter in all treated plants, but also that the curvatures were larger, which makes for a much sharper curved zone.

Discussion.—The experiments just described give a satisfactory explanation for the facts reported in the first part of this paper. In general it can be stated that applied auxins or hemi-auxins change the sensitivity of growing cells to auxin when measured as growth in length. This effect is an increase in sensitivity near the applied auxin and a decrease further away. This might be described in terms of rejuvenation and aging, as was done before (Went, 1935), but such terms do not give an explanation.

Table 6. Length of curved zone, total curvature, and curvature per mm. curved zone in Avena coleoptiles, if immediately after decapitation agar blocks soaked in 0.2 mg./l. indole acetic acid are unilaterally applied to the cut surface. Treatment of coleoptiles in the two hours previous to decapitation indicated in first column. Curvatures measured after 90 minutes.

	Afterwards 0.2 mg. indole acetic acid/l. produces					
Substance previously applied in lanoline to tip	Length of curved zone	Curvature	Curvature per mm. curved zone			
Pure lanoline	8.0 mm.	12.9°	1.6°/mm.			
Indole acetic acid 1 mg./g	7.9 mm.	14.3°	$1.8^{\circ}/\mathrm{mm}$ .			
Indole acetic acid 0.2 mg./g	7.4 mm.	13.4°	$1.8^{\circ}/\mathrm{mm}$ .			
Cis-cinnamic acid 10 mg./g	$6.7 \mathrm{\ mm}$ .	17.6°	2.6°/mm.			
Phenyl acetic acid 2 mg./g	7.0  mm.	15.3°	$2.2^{\circ}/\mathrm{mm}$ .			
Phenyl butyric acid 2 mg./g	$6.2 \mathrm{mm}.$	14.4°	$2.3^{\circ}/\mathrm{mm}$ .			

To understand the effect of auxin on growth it has been necessary to assume the interaction between auxin and the food-factor complex (Went, 1928, 1935). To this food-factor complex belong those factors or substances with which the auxin has to cooperate, or react, to cause cell elongation. One of the food factors was identified as sugar by Schneider (1938). From the work of Mitchell and Martin (1937) and many others it appears that the application of indole acetic acid may affect the rate of carbohydrate transfer. However, this transfer has not been shown to occur previous to the morphologic responses, so that they may be ascribed to the result rather than the cause of the increased growth or root formation. In the experiments which are described here the food factor affected is not sugar, since the application of sucrose did not change the results.

Now the main fact established in this paper is that under the described conditions the reactivity of the cells near the applied auxin tends to increase, whereas further away we find a simultaneous decrease, which is of the same order of magnitude. Since we ascribe the sensitivity of the cell to its food factor content, the conclusion seems logical that under the influence of apically applied auxin this food factor is redistributed, so that it moves toward the auxin supply. This leaves a (temporary) deficit in the lower zones, which is offset as soon as the downward moving auxin has reached the source of the food factor (the seed, see Went, 1935).

Thus we see that all facts line up nicely in a general scheme when we recognize that the effect of apically applied auxin on cell elongation is two-fold. In the first place it causes or at least increases the upward transport of certain substances, necessary for cell elongation, such as the "food factor" in Avena. This effect is produced even by hemiauxins, which are substances unable by themselves to cause growth, such as cyclohexane acetic acid and  $\gamma$ -phenyl butyric acid. And in the second place they can specifically induce cell elongation in the presence of the other cell elongation substances. A very clear distinction between these two properties of the same substance is possible in the case of phenyl acetic acid. Since its growth effect is exerted in high

concentrations only, whereas the translocation effect apparently occurs in lower concentrations as well, a regional separation between the two processes is possible—a fact which is difficult to establish for the most active auxins. In the first two hours after apical application of phenyl acetic acid it causes the food factor present in the lower zones to move upward and collect in the upper zones of the coleoptile. Since during that period its effect apparently does not reach farther downward, inducing an increased supply from the seed, there is, at least temporarily, a food-factor deficiency in the lower zones, demonstrating itself in a growth inhibition. The increased food-factor supply near the tip, together with the growth promoting effect of the high phenyl acetic acid concentration, will cause a growth acceleration of the highest zones.

In these cases it has been clearly demonstrated how, through two completely different physiological activities, one substance may be promoting growth in one part of the plant and inhibiting it in another. There is not the slightest indication that concentration differences are instrumental in these qualitatively different growth responses, since in isolated pieces of tissue the effect on the different zones was always the same, independent of the concentrations. It has been sufficiently proven that in the underlying case the inhibition is *indirect* and is a correlation phenomenon. It also is clear that if a substance lacks the growth property, it still may induce a growth inhibition, since the inhibition is not exerted through extra growth somewhere else. The use of chemically different pure substances has been of great help in solving this problem, since in addition to the physiological differentiation in time and place of the activities of auxin, even a complete separation of the translocation and growth activities with different substances was possible.

In conclusion one might ask what importance these experiments have in elucidating normal growth. The substances used in this investigation are partly not naturally occurring in plants, and besides, the concentrations employed are far beyond what normally would occur. The main point is that by affecting the movement of other growth factors, inhibitions may be caused by growth promoting sub-

stances. This same type of growth inhibition was shown to occur in lateral bud inhibition (Went, 1939a), so that these two cases mutually confirm each other. It is believed that the naturally occurring auxins affect growth in the same way as described above for the high concentration of phenyl acetic acid, for example. This accounts then for the food factor moving toward the growing point of a stem and for the decreased auxin sensitivity of the lower zones of the growing stem, where aging occurs. A similar case of auxin affecting the translocation and accumulation of other growth factors was described in the case of root formation by Cooper (1938) and Went (1938). A further discussion of this problem will appear elsewhere.

APPENDIX.—In this connection another growth inhibition should be discussed. Recently Snow (1937) described two new growth promoting substances. In another connection (Koepfli, Thimann, and Went, 1938) it has been shown recently that for at least one of these substances, dibenzoyl peroxide, this claim is erroneous. If this substance is mixed with lanoline and smeared along one of the sides of an Avena coleoptile, the coleoptile will continue to grow straight for about half a day, but then shows a tendency toward a positive curvature, as far as the lanoline extends along the coleoptile. This also seems a case of growth inhibition, and the substance was investigated in a section-growth test. Table 7 shows the results. Ten coleoptile sections of

Table 7. Growth in mm. of 4.2 mm. Avena coleoptile sections. First treatment 17 hours, second treatment next 24 hours.

First treatment		Second treatment, all in indole acetic acid 1 mg./l.
Indole acetic acid 1 mg./l	1.48	0.06
Benzoyl peroxide satd	0.52	0.11
Water	0.51	1.42

4.20 mm. length were placed in each of the following solutions: water, a saturated dibenzoyl peroxide solution, and indole acetic acid 1 mg./l. In the first 17 hours there was a good growth effect in the auxin solution, but growth in water and in dibenzoyl peroxide was the same. This proves that this substance is no growth promoting substance at all. For the next 24 hours all sections were transferred to an indole acetic acid solution 1 mg./l. Those previously in auxin hardly grew at all; all food factor had

been used up in growth in the previous period. Those coming from water grew a fair amount, proving that unused food factor was still present in the sections. But the sections coming from the dibenzovl peroxide did not grow any more. Thus under the influence of this substance the food factor seems to have disappeared, probably by destruction. Since the growth in the first period was not affected, it hardly can have affected the auxin. Besides, if it did do so, a more immediate response in the bending test could be expected. There were also no indications that the lack of growth was due to toxicity, the cells remaining turgid. Thus it seems that in dibenzoyl peroxide we possess a substance which is directly inhibiting growth through its effect on the food factor.

#### SUMMARY

Occasionally after application of high concentrations of indole acetic acid, but regularly with phenyl acetic, cyclohexane acetic, or  $\gamma$ -phenyl butyric acids, a growth inhibition of the lower zones of an Avena coleoptile occurs. This inhibition is demonstrated by direct growth measurements, both of whole coleoptiles and of zones of intact plants, but more easily by unilateral application of these substances to the cut surface of decapitated coleoptiles (Avena test), when they show positive curvatures within  $2\frac{1}{2}$  hours after application.

This growth inhibition is an indirect effect of these substances, observable only in intact or decapitated plants, but absent in short coleoptile or pea stem sections. Experiments have indicated that the inhibition is due to removal of other growth factors (in the Avena coleoptile the food factor) from the zone of inhibition, which are then accumulated near the place of application of the active substances (the tip). Thus it is clearly proven that the effects of substances like phenyl acetic acid (and by inference also for indole acetic acid) on growth are due to a dual action; in the first place they redistribute other growth factors, and in the second place they react with those other factors to give cell elongation. Some substances (cyclohexane acetic acid, γ-phenyl butyric acid) possess only the property of redistributing the other growth factors without directly affecting cell elongation.

In one case, dibenzoyl peroxide, experiments indicated that the growth inhibition caused by this substance is probably due to its effect on the food factor.

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## A NEW FUNGUS WITH ANTERIORLY UNICILIATE ZOOSPORES: HYPHOCHYTRIUM CATENOIDES <sup>1</sup>

### J. S. Karling

In a series of attempts to inoculate bits of young Zea mays leaves with Physoderma zeae-maydis in Petri dishes for the purpose of securing the ephemeral zoosporangia of this chytrid, another unusual fungus appeared in the trichomes along the leaf margins. The origin of this fungus is uncertain, but it may have been introduced with the inoculum of Physoderma resting spores. In these experiments young unfolded and delicate leaves of corn were washed in several changes of distilled water, and strips of the leaf margins with trichomes were then placed in Petri dishes partly filled with sterile charcoal water. To these was added a dense suspension of *Physoderma* resting spores. Approximately ten days later, after the leaf margins had died and were being attacked by protozoa and bacteria, the new fungus made its appearance in the trichomes and soon spread to adjacent epidermis and parenchyma cells. So far, zoospore infection of normal healthy trichomes in which the protoplasm was still streaming has not been observed, but young thalli have been found in palisade cells of which the plastids were still green and appeared to be normal. Attempts to infect living and dead trichomes of cucurbits, tomatoes, and potatoes, root hairs of wheat, barley, and rve, as well as cooked filaments of Cladophora and Oedogonium have failed. The fungus, however, will infect and grow well in cooked internodes of Chara and Nitella. It is apparently parasitic to some degree but grows best in dead cells and on synthetic nutrient media.

Bits of heavily infected corn leaves were transferred to 1 per cent potato-dextrose and corn meal agar, and within four days the fungus had spread onto and made slight growth in these media. Transfers were made at once, but, since the fungus grows very slowly, it has been difficult to free it from bacteria. The addition of 4.68 per cent potassium thiocyanate, according to Bojanovsky's (1938) method, to corn meal and dextrose agars has retarded the growth of bacteria to some extent, but so far it has not proven of much value in securing pure cultures.

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Cultures on agar when flooded with a thin film of sterile charcoal water form zoospores in a few hours which disperse very rapidly. These come to rest and germinate as the water evaporates, and it is thus possible to pick up isolated spores and establish pure monospore cultures. So far, this fungus seems to grow best on 1 per cent corn meal and potatodextrose agars, which are sufficiently soft to permit penetration and spread of the thallus.

In trichomes of corn this fungus is often strikingly like Catenaria except for the lack of rhizoids, while in parenchyma cells and on agar it may frequently resemble Myzocytium. It differs fundamentally from species of these genera, however, by the presence of a single anterior cilium on the swarmspores. This character, together with the similarity in structure of the thallus and the lack of rhizoids, seems to indicate a close relationship with Hyphochytrium as this genus is now known. For the time being I am accordingly including my fungus in this genus and designating the species as catenoides with the purpose of emphasizing the general appearance of its thallus.

HYPHOCHYTRIUM catenoides Nov. sp.—Thallus predominantly polycentric and intramatrical; usually hypha-like, and consisting of a linear series, up to 500  $\mu$  in extent, of intercalary and terminal swellings and sporangia connected by tubular hyphae or isthmuses 1 to 38  $\mu$  in length and 2.2 to 4  $\mu$  in diameter; occasionally monocentric, oval, elongate, spherical and Olpidium-like. Zoosporangia terminal or intercalary, delimited by cross septa, hyaline, smooth, spherical, 10–35  $\mu$ , oval, 10 $\times$ 12  $\mu$ –18 $\times$ 22  $\mu$ , broadly spindle-shaped  $6\times10~\mu-9\times18~\mu$ , elongate and sometimes slightly irregular with 1-4 single or branched, straight, curved, coiled, or irregular exit tubes,  $5-250 \mu$  in length and  $3-6 \mu$  in diameter. Contents of sporangium usually emerging as a naked spherical mass on the outside and undergoing cleavage into swarmspores; occasionally undergoing complete or partial cleavage in the sporangium whereafter the segments glide out in succession. Zoospores anteriorly unicilate, slightly flattened, oval, and elongate,  $1.5\times3.5~\mu-2\times3~\mu$ , with several small, slightly refractive granules. Sexuality and resting spores unknown.

Weakly parasitic and saprophytic in Zea mays, Nitella flexilis, and Chara coronata in New York

HYPHOCHYTRIUM catenoides Nov. sp.—Thallo praecipue polycentrico atque intramatricali; semper fere hyphae simili, constato ordine, usque 500  $\mu$ , intercalarium atque terminalium tumorum et sporangiorum tubularibus hyphis vel isthmis 1-138 μ long.  $\times$  2.2-4  $\mu$  dia. conjunctorum; aliquando monocentrico, ovato, longius facto, globoso, Olpidio simili. Zoosporangiis terminalibus vel intercalaribus, parietibus disjunctis, hyalinis, levibus, globosis  $10-35 \mu$ , ovatis  $10\times12 \mu-18\times22 \mu$ , fusiformibus  $6\times10~\mu-9\times18~\mu$ , longius factis atque aliquando leviter irregularibus, uno usque quattuor, solis vel ramosis, directis, flexis, tortuosis, vel irregularibus tubulis dimissionis, 5–250  $\mu$  long. atque 3–6  $\mu$  dia. praeditis. Quibus continet sporangium cumulo nudo globoso semper fere extra emergentibus, et zoosporis ruptis; aliquando absolute vel aliquatenus sporangio ruptis, segmentis ordine inde elapsis. Zoosporiis cilio anteriore praeditis, planis, ovatis, et longius factis,  $1.5 \times 3.5 \mu - 2 \times 3 \mu$ , multis parvis refractis globulis praeditis. Sexualitate atque sporis perdurantibus incompertis.

STRUCTURE AND DEVELOPMENT OF THE THALLUS. -The thallus of H. catenoides is usually polycentric, hyaline and greyish-granular in color, and consists of linear series of spherical, oval, broadly spindle-shaped or fusiform swellings and sporangia connected by short isthmuses or tubular hyphae of variable lengths. Figure 15 shows an unusually extensive, branched thallus from a large trichome of corn with ten swellings and sporangia in various stages of maturity. In such host cells the appearance of the thallus is usually very different from that in agar cultures. It is usually less branched and crowded and more often consists of a single row of rather widely separated sporangia connected by long, tubular, septate, hyaline, and empty hyphae of fairly uniform diameter, as is shown in figure 16. This particular thallus measured 500  $\mu$  in over-all length. The length of the connecting hyphae may vary from 10 to 138  $\mu$ , and in some instances they are reduced to mere isthmuses,  $1-5 \mu$  in length. They are usually fairly uniform in diameter, 2.2  $\mu$ - $4 \mu$ , but in some instances they may be rather irregular and much thicker. This is particularly evident in the extensions emanating from sporangia B and D of figure 15. Such structures are very common in agar cultures and may later mature into elongated irregular sporangia. The septa are fairly distinct and extend completely across the hyphae. Numerous tests with chloro-iodide of zinc have been made on the entire thallus, but no cellulose reaction has been observed.

The thalli may occasionally be very much shortened and crowded in the small trichomes and have the beaded appearance shown in figure 17, which is very similar to that of *Myzocytium* species growing in filamentous algae. In such thalli the connecting isthmuses or hyphae may be lacking altogether, so that the sporangia are separated only by transverse septa at the constrictions. Reduced monocentric thalli of the type shown in figure 18 may also occur, but they appear to be comparatively rare in cells or trichomes of corn. On agar, however, they may be very abundant and appear to be nothing more than quiescent spores which have enlarged into spherical or oval sporangia. In these cases the empty hyphal arms, such as those shown in figures 15A and 18, may be lacking, and such thalli are strikingly similar to those of Olpidium. In trichomes of small diameter these spherical monocentric thalli may expand to the limits of the side walls, and any further growth must accordingly occur in a linear direction. As a result the thalli become elongate and cylindrical with rounded ends, and completely fill local portions of the small trichomes. It is to be noted in this connection that monocentric thalli similar to those reported above have already been described by Valkanov (1929) in H. Hydrodictii. In soft agar the thallus may grow more or less radially so that the colony may become globular or almost spherical in outline. A more detailed account of its growth and appearance in such media will be given later in connection with a cytological study of nuclear and cell division, development, and organiza-

The development of the thallus can be followed very easily in the hyaline, almost transparent trichomes and on thin films of agar. The zoospore germinates readily in water and on the surface of the host and forms a germ tube which pierces the host wall and then swells up to form the primary enlargement or sporangium. In old thick-walled trichomes the hole or canal in the wall made by the penetrating tube is usually quite evident, as is shown in figures 11, 12, and 13. So far, no persistent zoospore cases have been observed as in H. Hydrodictii. Figure 5 shows an early germination stage in water outside the host cell. Sometimes two and three germ tubes may develop, as is shown in figure 6. These tubes are usually fairly thick, hypha-like, and blunt at the apex. Figure 7 shows the germination of one of the large multiciliate zoospores which often occur in this species. In figure 8 most of the protoplasm has passed down into the germ tube, leaving the zoospore case partly empty, while the primary enlargement is forming as a swelling of the tube. This does not always occur, however, since in agar cultures particularly, as has been noted above, the zoospore itself may frequently grow directly into the primary enlargement or sporangium (fig. 9). Sometimes the primary enlargement may become elongated and irregular like the one from a trichome of corn shown in figure 10.

In figures 11 to 14 are shown successive developmental stages of a thallus within a trichome of Zea mays. The earliest stage (fig. 11) appeared as a globule lying against the inner layer of the cell wall, but there was no evidence of a germ tube or empty zoospore case on the outside, although the penetra-

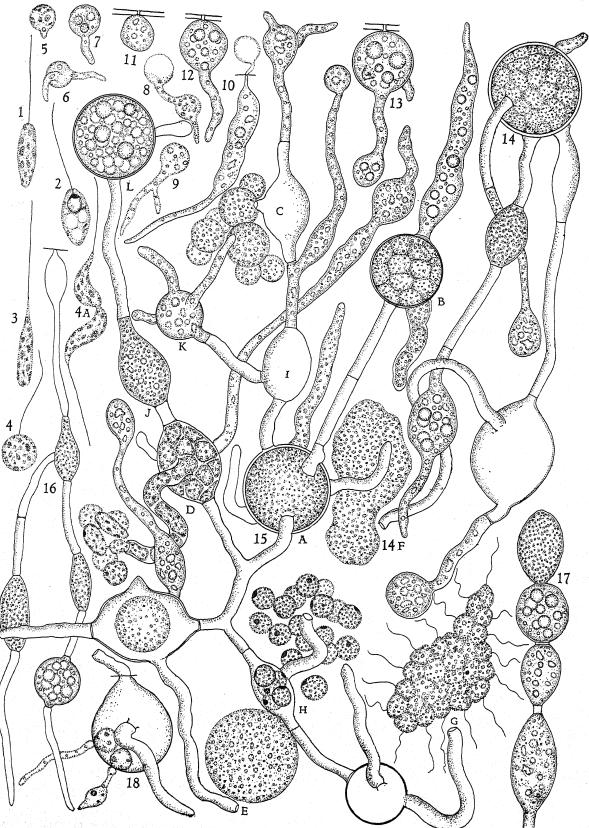


Fig. 1-18.

tion pore was quite visible in the wall. Even at this early stage numerous oval and irregular granules occur in the cytoplasm, but they do not appear as glistening and refringent as those present in the majority of the chytrids. The protoplasm as a whole has a different appearance from that of most rhizidiaceous chytrids and looks more like that of Pythium. Figure 12 shows the thallus four hours later. The primary enlargement has doubled in size, while the more refractive material of the cytoplasm has greatly increased in amount. On the under side a thick tubular hypha has grown out. Five hours later the thallus had the appearance shown in figure 13. The initial hypha has begun to enlarge at its tip and thus has formed the rudiments of a second enlargement, while two additional hyphae are forming on the primary enlargement. The number of hyphae which grow out from a single enlargement may vary from one to ten, but not all of them reach great length or continue to grow. Oftentimes their protoplasm is retracted into the enlargement again, and they are then delimited by cross walls, leaving them hyaline and empty. Two such short empty hyphae are shown at the right and left of the sporangium in figure 15A and one each in figures 15L and 18.

Within thirty-two hours the thallus had developed to the extent shown in figure 14. The primary enlargement has become a sporangium with a short exit tube, and its content has undergone cleavage into zoospore initials. One of the hyphae has formed a large egg-shaped sporangium with a curved exittube, which has emitted its contents in preparation for zoosporogenesis (fig. 14F). The initial hypha has formed two enlargements, while a terminal one is forming on the third hypha. This thallus had a limited growth, and eventually all the enlargements were transformed into zoosporangia. It none the less illustrates clearly the characteristic type of growth of this fungus. As the intercalary enlargements and incipient zoosporangia develop, the protoplasm usually accumulates in them, leaving the tubular hyphae empty and delimited by cross walls. From the enlargements secondary, tertiary, etc., hyphae grow out, and the same type of structure and organization is continually duplicated. The rudiments of the enlargements and zoosporangia usually begin as swellings at the tip of the hyphae. Their origin is thus terminal, but as new hyphae grow out from them, they become intercalary in position. As far as my observations go at present, most of the enlargements are usually transformed into zoosporangia, but occasionally the protoplasm of the primary and secondary enlargements may pass on completely into the hyphae, leaving them empty, as is shown in figures 14, 15I, and 16. In such cases they are strikingly similar to the intercalary "Sammelzellen" of the Cladochytriaceae. The hyphae may also oftentimes branch at or near the apex, which leads to the development of a thallus like that shown in figure 15.

True proliferation of the sporangia occurs very rarely, if at all, according to present observations. Quite frequently, however, one or more zoospores which have failed to emerge may enlarge and develop into monocentric olpidiaceous thalli within the old sporangia, and such appearances may easily be mistaken for true proliferation. As I have pointed out elsewhere (Karling, 1937) in relation to other chytrids, however, this does not constitute true proliferation but is merely germination in situ. Germination in situ in the manner shown in figure 18 is not

uncommon in this species.

Sporogenesis.—The sporangia are terminal or intercalary and vary from spherical, 10-35  $\mu$ , oval,  $10\times12~\mu$ -18 $\times22~\mu$ , broadly fusiform,  $6\times10~\mu$ - $9 \times 18 \,\mu$ , to elongate and slightly irregular in shape. During maturation they develop one to four, single or branched, straight, curved, coiled, or irregular exit tubes which may vary from 5 to 250  $\mu$  in length and 3 to 6  $\mu$  in diameter. The very long exit tubes are usually developed when a thallus is transferred from agar to a hanging drop of water or nutrient solution. Under such conditions they may sometimes elongate to such an extent that most of the protoplasm in the sporangia is expended by their growth. As a result, the protoplasm may become so vacuolate and spent that no zoospores are formed. While exit-tube development is going on, the coarsely granular and somewhat refractive protoplasm begins to change in appearance and becomes more finely granular and glistening-grey in color. This change seems to take place as the result of an increase in dispersion of the more refractive material. It may remain in this condition for several hours, but shortly before the protoplasm escapes from the sporangium the small granules usually begin to undergo Brownian movement. This movement extends up into the exit tubes and seems to increase in intensity with time. In a few minutes the tip of the tube deliquesces, and the protoplasm streams out rapidly to form a naked spherical mass, as is shown in figure 15E. The emergence of the protoplasm occupies from 15 to 32 seconds, depending primarily on the size of the sporangium. Sometimes a portion of the protoplast may be left behind in the sporangium where it usually rounds up into a spherical mass.

The protoplasmic mass which emerges is entirely naked, and so far I have failed to observe the presence of a vesicular membrane as in Lagena, Pythium, etc. The Brownian movement of the granules usually continues for a short time, but gradually slows down and ceases within 65 to 98 seconds. Before this ceases, however, the mass begins to change, and within 30 to 50 seconds it may have become somewhat irregular in shape (fig. 14F). Within 120 to 150 seconds the first evidence of cleavage furrows appears, and after the lapse of 156 to 180 seconds since the mass first emerges, the cleavage segments begin to show individual movement and to glide upon each other. The cilia on the segments appear within 200 to 250 seconds and stand out more or less radially from the mass of segments (fig. 15G). They begin to wave back and forth at once, and as a result the whole mass begins to vibrate and oscillate. The zoospore initials continue to glide upon each other slowly and tug to get apart, and within 420 to 475 seconds they separate and swim away. The emergence of the protoplasm and the whole process of zoospore formation may take less than eight minutes. The zoospores may often fail to swim away, and after a few minutes of wiggling about they come to rest and round up in a loose mass near the exit tube, as is shown in figure 15H. Such masses closely resemble those of Achlya, but although I have kept them under observation for several days, I have so far failed to find any evidence of diplanetism.

Oftentimes cleavage may be unequal and incomplete, so that irregular bi-, tri-, quadriciliate, etc., masses are formed. Uniciliate segments may be partially delimited in such masses and then ensues an active tug of war on the part of the units to free themselves. One may thus frequently find 2, 3, 4, and 5 segments straining in opposite directions to get apart. They often succeed in doing so and swim away, but, failing to do so, they frequently unite again into a large spherical mass after a long period of activity. In cases where two segments are involved they may often give the misleading appear-

ance of fusing gametes (fig. 4A). The method of sporogenesis described above appears at present to be the most common and normal type, but several variations of the process have been observed. If the exit tube fails to open, the content of the sporangia may undergo cleavage into segments, as is shown in figure 15B. Under such conditions the segments are usually so tightly packed together that little or no movement is visible. The segments soon round up and may remain in this quiescent condition for several days before disintegrating. Oftentimes the exit tube does not open until after cleavage within has been completed, and then the individual zoospores glide out one by one, as in figure 15D. As the pressure is thus released, those still within may swarm very rapidly. Sometimes cleavage may be only partially completed when the exit tubes open, and in such cases individual zoospore initials and large uncleaved masses of protoplasm may emerge in succession. Such segments and masses usually round up near the mouth of the exit tube without becoming actively motile or undergoing complete cleavage. They may persist in this state for several days and then germinate (fig. 7) or finally disintegrate. The large masses are doubtless multinucleate, but I have not stained any of them to determine whether or not the nuclei fuse before germination occurs.

It is obvious from the above account that the process of sporogenesis varies markedly in H. catenoides, and in this respect it is more similar perhaps to species of Lagenidium and Myzocytium than to Hyphochytrium as it is now known. In H. infestans and H. Hydrodictii, Zopf and Valkanov describe the zoospores as being completely formed in the sporangia, emerging singly in succession, and swimming directly away. In H. catenoides, on the other hand, as has been noted above, such a method of sporogenesis and subsequent behavior of the zoo-

spores appears to be exceptional as far as my observations go at the present time.

ZOOSPORES.—The zoospores are oval and somewhat flattened in shape, and as a consequence, they appear quite different in optical and side views. Figures 1 to 4 show zoospores in both views and in various stages of motility. In side view they have the appearance shown in figure 1 with a blunt or tapering anterior and a more slender posterior end, and in this view they measure  $1.5 \times 3.5 \,\mu$ . Oftentimes they are even more drawn out so as to appear as rods about three times as long as broad. Figure 2 shows a fixed and stained swarmspore in optical view,  $2\times3$   $\mu$ , with a well-defined nucleus and several conspicuous vacuoles. In the protoplasm of living zoospores numerous small granules are present which imparts a greyish, granular, and somewhat glistening appearance to the swarmspores as they swim about. These granules appear to become fewer in number toward the end of the motile period, as if some of the smaller granules had coalesced to form 4 to 8 larger ones.

The most striking structure of the zoospores, however, is a single, 7 to 10  $\mu$  long, conspicuous, anteriorly attached cilium which is directed forward as the spores swim about. During the active swimming stage the spores move so rapidly that the cilium is usually invisible in transmitted light, but in a ½ per cent agar or a syrupy gelatin medium the movement of the spore is retarded to such an extent that the position and action of the cilium may be readily observed. Under such conditions, it may extend out more or less straight in front with the end lashing back and forth. The zoospore thus moves forward in a fairly straight path, and when the cilium comes into contact with an object, it gives a vigorous lash which causes the spore to back out. It then starts off in another direction. This backward movement of the zoospore under such conditions is very characteristic, and from observing it, one might get the impression that the cilium is posterior, but when the swarmspore is swimming steadily forward, the cilium can be clearly seen out in front. Actively swimming zoospores have frequently been fixed with osmic acid and stained in toto to determine the presence or absence of a second shorter cilium. Occasional large isocont bi-, tri-, and quadriciliate spores have been found, but these appear to have arisen by unequal cleavage. More than 95 per cent of the fixed and stained zoospores so far studied have been uniciliate, and no second, shorter cilium has been observed. While moving forward, the zoospore may frequently roll over, presenting optical and side views in alternate fashion. In a less viscid and more watery medium this rolling motion may often be even more conspicuous. In water mounts the zoospores change direction so frequently and rapidly that their movement may often appear to be a darting one. It none the less appears to be quite different from that of most chytrids.

In dilute agar media and gelatine such as described above, the zoospores may often become amoeboid and undergo conspicuous contortions and

changes in shape. The anterior end may be drawn out into a tapering filament as is shown in figure 3. In other instances pseudopods may push out at various places on the periphery. The period of active motility usually varies from 20 to 80 minutes according to present observations, but in exceptional cases it has lasted as long as 3 hours. As the zoospores begin to slow down they become more globular in shape, and after coming to rest, they are usually spherical, as is shown in figure 4. At this stage the cilium may continue to vibrate for a few minutes, imparting a slight oscillating movement to the body of the spore. This movement becomes slower and slower and eventually ceases.

It may be worth while to note here that Rhizo-phlyctis Petersenii and Nowakowskiella elegans have been found in abundance in bits of corn leaves during the course of this study. The latter has been isolated in pure culture on corn meal agar and is now growing vigorously. On this medium the rhizo-mycelium becomes very extensive and comparatively coarse.

Discussion.—Whether or not H. catenoides is identical with H. infestans or H. Hydrodictii will not be certain until the latter species have been studied more intensively and the life cycles of all three are fully known. At present, however, H. catenoides shows several distinctive differences. Its zoospores are elongate, oval, and slightly flattened instead of spherical, as in the other species, and do not possess a single, highly refractive globule. In addition, the normal and more usual method of sporogenesis is quite different from that reported for H. infestans and H. Hydrodictii. Furthermore, the presence of elongated exit tubes in H. catenoides is another striking difference, but whether or not this character is of fundamental significance is not vet certain. None the less, I believe these differences are sufficient to warrant the establishment of a new species.

In the event H. catenoides proves to be a valid species of Hyphochytrium, its discovery confirms Zopf's (1884) and Valkanov's (1929) observation on this genus and their demonstration of the existence of a group of fungi with hypha-like thalli and anteriorly uniciliate zoospores. This genus was established by Zopf for a parasite on the ascocarps of Helotium, which is characterized primarily by anteriorly uniciliate zoospores and a non-rhizoidal mycelioid thallus with terminal and intercalary swellings and sporangia. Since that time Hyphochytrium, as well as all other genera, Latrostium, Reesia, etc., in which anteriorly uniciliate swarmspores are reported to occur, have been regarded with considerable doubt and skepticism. Vuillemin (1908) expressed the belief that H. infestans is nothing more than a hypha of another fungus parasitized locally by a monocentric olpidiaceous chytrid. Minden (1915) is inclined to agree with Vuillemin and changes the genus name to Hyphophagus in order to avoid confusion in relation to his restricted usage of the family name Hyphochytriaceae. In 1929, however, Valkanov found another species, H. Hydrodictii, parasitizing young cells of Hydrodictyon, and his observations thus placed the genus, as defined by Zopf, on a firmer foundation. Mycologists since then have, none the less, either looked upon it as a doubtful genus (Fitzpatrick, 1930) or disregarded it completely (Gwynne-Vaughan and Barnes, 1937). My observations lead me to believe that Zopf's genus is valid and that distinct provision must be made in our classification for a group of fungi with swarmspores of this type.

Like most algologists and mycologists, I am strongly of the belief that the number, position, and relative lengths of the cilia are fundamentally significant in classification and phylogeny, and also indicative of relationship, and for this reason I do not believe Hyphochytrium can be included in a family other species of which are characterized by posteriorly uniciliate zoospores. I am accordingly proposing the establishment of a separate family for Hyphochytrium and other similar fungi which may possibly be found in the future. Since Fischer's (1892) family Hyphochytriaceae already bears the same prefix as in Hyphochytrium, I propose, in the interests of clarity, to adopt and restrict it rather than suggest a new family name.

The name Hyphochytriaceae has been variously used in the literature, and very few mycologists are agreed as to what genera should be included in it. Fischer used this name synonymously with the Cladochytriaceae, but Schroeter (1897) distinguished it from the latter and limited it to Catenaria, Hyphochytrium, Polyrhina, and Tetrachytrium. Lotsy (1907) included only Hyphochytrium in this family, but he changed the spelling to Hyphochytridiaceae. Minden (1915), on the other hand, excluded Hyphochytrium (Hyphophagus) entirely and limited the family to Macrochytrium and the two doubtful genera, Tetrachytrium and Zygochytrium. Gäumann (1926), Gäumann and Dodge (1928), and Gwynne-Vaughan and Barnes (1937) restricted it to Macrochytrium alone, while Fitzpatrick (1930) did not recognize the family as being valid. Bessey (1935) followed Minden's viewpoint with the exception that he included Hyphochytrium, and placed the family Hyphochytriaceae among the Monoblepharidales. It is thus obvious that there is but little agreement as to the limits of this family and what genera are to be included. In view of this, a further change will not violate any well-established and generally agreed upon precedence of family name, and I thus propose to restrict the Hyphochytriaceae to species having non-rhizoidal, hypha-like or mycelioid, predominantly polycentric thalli with terminal and intercalary swellings, sporangia, and resting spores, and anteriorly uniciliate swarmspores. In this sense the family now includes one genus and three species.

For species with similar thalli and posteriorly uniciliate zoospores, I suggest the family name Myceliochytriaceae. This family would accordingly include at present Coenomyces Deckenback (1903), Megachytrium Sparrow (1931, 1933), and possibly Macrochytrium Minden (1902, 1923). On the

basis of thallus structure, Tetrachytrium and Zygo-chytrium might be listed provisionally in this connection as of very doubtful relationship, but at present this would be highly questionable, because of the types of sexuality which they exhibit. For the time being and in our present state of knowledge concerning the chytrids, the Myceliochytriaceae may perhaps be regarded as the climax family of the chytridiaceous species on the basis of size, complexity, structure of the thallus, and its similarity to the mycelium of the higher fungi. The idea that this family may be a connecting link with the Blastocladiales is very suggestive and stimulating, but so far we have but little supporting evidence.

As to where the family Hyphochytriaceae, in the restricted sense used in this paper, belongs among these simpler fungi is of course uncertain at the present time. Students of the Chytridiales are inclined to regard this so-called order as a provisional and convenient dumping-ground for a heterogeneous group of relatively simple fungi, or to limit it to a comparatively small and simple species with posteriorly uniciliate zoospores and exclude those with biciliate swarmspores. The latter students would accordingly exclude the family Woroninaceae and Ectrogellaceae as close relatives possibly of the Lagenidiales<sup>2</sup> and Saprolegniales and designate the members of the Olpidiaceae, Synchytriaceae, Rhizidiaceae, and Cladochytriaceae as "true chytrids" of definite relationships. While I agree that there are at present no very good grounds for linking the latter four families with the former two, none the less I believe that our knowledge of this so-called order as it now stands is still too meager to draw any definite and final conclusions as to phylogenetic groups. With the increasing interest in these relatively simple fungi, new genera and species will doubtless be discovered which may possibly upset our current opinions completely. Furthermore, since our present system of classifying the chytrids is

<sup>2</sup> Since Miss Berdan (1938) has shown that *Ancylistes* no longer relates to this group, the ordinal name Ancylistales is not tenable. I therefore suggest raising the family Lagenidiaceae to ordinal rank for the time being until its relationship becomes better known.

based largely on vegetative characters, it is to a large degree artificial and will naturally have to be modified as the types of sexual reproduction in the group become better known. For these reasons I regard the so-called order Chytridiales at present as a heterogeneous group of comparatively simple species which may indicate several lines of relationship and am including the family Hyphochytriaceae as a member of this order.

#### SUMMARY

Hyphochytrium catenoides has been found as a weak parasite and saprophyte in trichomes and parenchyma cells of Zea mays and in cooked internodes of Chara and Nitella. It is characterized primarily by anteriorly uniciliate zoospores and a predominantly polycentric, non-rhizoidal, hypha-like thallus with terminal and intercalary swellings and zoosporangia connected by tubular isthmuses or hyphae of varying length. In sporogenesis the contents of the sporangia usually emerge through exittubes of variable length as a naked mass and undergo cleavage into zoospores on the outside without the formation of a hyaline vesicular membrane. Oftentimes, however, partial or complete cleavage may occur within the sporangia, and in such cases the zoospores may frequently swarm first within the sporangium, then emerge in succession, and swim directly away. So far, no evidence of sexuality or resting spores has been found.

The discovery of this species supports Zopf's and Valkanov's observations on the genus Hyphochytrium and their demonstration of the existence of hypha-like fungi with anteriorly uniciliate zoospores. It is accordingly proposed that the family Hyphochytriaceae be restricted to fungi of this type. Another family, Myceliochytriaceae, is suggested for similar chytridiaceous fungi with posteriorly uniciliate zoospores. This latter family would include at present Coenomyces, Megachytrium, and possibly Macrochytrium.

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### THE CYTONUCLEAR RATIO IN DEVELOPING PLANT CELLS 1

Vivian V. Trombetta

MUCH WORK has been done on the problem of the nucleocytoplasmic ratio in plant cells, but although the literature is very extensive, the results still remain confusing and often contradictory. The purpose of the present study was twofold—to determine the quantitative relationship of nucleus to cell in meristematic plant cells and to show how this relationship is altered as these meristematic cells de-

velop to their mature condition. The problem of the ratio of nuclear volume to cell volume has been studied extensively in both plants and animals, and in many cases definite relationships have been found to exist. Sachs (1892, 1893) and Strasburger (1893) noted the relative minuteness of meristematic cells and concluded that the working-sphere of the nucleus must be a very restricted one. Strasburger obtained a ratio of 2:3 for the average diameters of nuclei to cells in terminal meristems, measurements which led him to believe that a very close correlation exists between nuclear and cell size. Conklin (1902) showed that the relation of nucleus to cell is maintained in decreasing cell size in the cleavage of the ovum in annelids and gasteropods, where marked inequalities of division are frequently found; although the daughter nuclei are equal in size after division, they grow to a size which is roughly proportional to that which the cell attains. Koehler (1912) found a very marked increase in the total volume of the nuclei in the cleavage of Strongylocentrotus, up to about the ninth cleavage, whereas the volume of the cytoplasm remained unchanged. Guillermond (1909) found that the nuclear diameter always remains approximately proportional to that of the cell in Endomyces fibuliger and Saccharomyces capsularis. Henneberg (1915) obtained similar results on Saccharomyces. Teissier (1927) demonstrated in oogenesis in Hydractinia echinata that the dimensions of the nucleus increase less rapidly than the dimensions of the cell, but that the relative rates of change of nucleus and cell are constant.

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The nucleo-cytoplasmic ratio has frequently been studied in cases of polyploidy. In 1902 Gerassimow obtained races of Spirogyra with double the original nuclear volume and found that the size of a cell is a function of the mass of its nuclear substance. Winkler (1916) also found the size of cells of giant tetraploid individuals of tomatoes and nightshades to be dependent upon nuclear size. Similar results were obtained from a study of Oenothera gigas by Gates (1909). Lindstrom and Humphrev (1933) found cell size and nuclear size to be related to chromosome number in tetraploid tomatoes. Boveri (1905) showed that the size of both nucleus and cell in echinoderm larvae is dependent upon the chromosome number and not merely upon the amount of chromatin contained in the nucleus; he concluded that it is the surface of the nucleus, rather than its volume, which is proportional to the number of chromosomes and that the size of the cell is proportional to nuclear surface and chromosome number. Erdmann (1908) concluded from investigations on sea urchins that chromosomal mass, rather than chromosomal number is related to cell size.

Several studies have been made on the relation of chromosome number, nuclear size, and cell size in polyploid mosses which have been produced by artificial apospory. The Marchals (1909) found the volumes of nuclei and cells of Amblystegium, Physcomitrium, and Funaria to be directly proportional to the number of chromosomes. Cells and nuclei of the tetraploids are approximately twice those of the diploid; those of the diploid, twice those of the haploid. Wettstein (1924) found an increase in cell size in polyploid mosses, but the increase was not directly proportional to chromosome number. Very little is said about nuclear size in Wettstein's work. If it is proportional to chromosome number, as Boveri and most other workers have found to be the case in other forms, then a definite relationship exists between cell size and nuclear size in mosses. The dimensions of the nucleus evidently increase less rapidly than the dimensions of the cell, but the relative rates of change of nucleus and cell are constant.

The polyploid relationships discussed thus far are those which have been found in *induced* polyploids. In such forms there appears to be a close correlation between chromosome number, nuclear

size, and cell size. In the case of *natural* polyploids, however, body size, organ size, and cell size are, in general, not very different from their normal size in diploids. Gaiser (1927) found a general lack of uniform relation between number of chromosomes, cell size, organ size, and plant stature.

The nucleo-cytoplasmic ratio has been looked upon as an important factor in senescence. Botanists, as well as zoologists, have attributed senescence and rejuvenescence to changes in this relationship. Minot (1908) maintained that with age the nucleus of a cell decreases in size and that as a result there is a decrease in the nucleo-cytoplasmic ratio. Hertwig (1903), however, believed that physiological depression, senescence, and natural death are associated with an increase in the relative size of the nucleus. The results of Hartmann (1919a) on Cladocera also support Minot's theory. Meyer (1917) tried to measure the volume of the cytoplasm in the palisade cells of Tropaeolum in an attempt to find whether the nucleo-cytoplasmic ratio changes with age and discovered the ratio to be less in an old yellow leaf than in a young green one. In a study of senescence in the cells of the potato plant, Lutman (1925) found that senescence is accompanied by an increase in cell size without corresponding increase in nuclear size.

The nucleo-cytoplasmic ratio has often been thought to be important in the process of cell division, the ratio having been suggested as a factor in initiating cell division (Godlewski, 1910). Hertwig (1908) explained nuclear and cell division by his concept of "Kernplasma-Spannung," a "tension" created by an increasing disproportion between nuclear volume and cell volume, in favor of the nucleus, as the cell enlarges. Popoff (1908) also attributed cell division to a similar tension created in cells. Boveri (1905) believed the disproportion to be one of nuclear surface to cell volume. From measurements of nuclei and cells in the primary meristems of a number of the higher plants, Abele (1936) believes that cell division follows a disproportion in the ratio of nuclear surface to nuclear volume. Conklin (1912) in his work on cleavage stages in Crepidula denied the existence of a constant and self-regulatory nucleo-cytoplasmic ratio, holding rather that changes in this ratio are not causes of such cell activities as division but results of the metabolic processes by which such activities are brought about.

A number of physiological factors have been found to affect the nucleo-cytoplasmic ratio. Rautmann (1909) showed that there is a change in the ratio with a rise and fall of temperature in Paramaecium, although the ratio does not parallel the temperature changes exactly. Marcus' work (1906) also supports this conclusion. Hartmann (1919b), on the other hand, found that in cells of the plerome of Zea Mays and Phaseolus coccineus and of the dermatogen of Pisum sativum, subjected to a temperature of from 11.5°C. to 42°C., the nucleo-cytoplasmic ratio becomes less with an increase in tem-

perature. A similar lowering of the ratio is produced by X-ray treatment. Goodspeed (1929) shows this to be the case in leaves of *Nicotiana*, the seeds of which were irradiated.

The nucleo-cytoplasmic ratio is frequently a very variable one. Conklin (1912) contends that such a ratio does not hold even in the cells of the same tissue, for it varies with the different phases of mitosis. In cells of the cambium of *Pinus strobus*, Bailey (1920) found that the ratio of cell to nucleus varies from 12:1 in ray cell initials to 286:1 in tracheid initials. Klieneberger (1918), Ensign (1919), and Tischler (1924) have reported similar variability.

Surprisingly little work has been done on developing plant cells. Such questions as whether the nucleus increases in size as a cell vacuolates and whether the ratio of nucleus to cell changes during development remain unanswered.

MATERIALS AND METHODS .- Any study of the nucleo-cytoplasmic ratio in mature plant cells is necessarily a difficult one. The large central vacuole serves to press the cytoplasm in a thin film against the wall. If this cytoplasmic layer were of an equal thickness throughout, its volume might be determined fairly accurately; but such is probably not the case. The situation is very unlike that found in meristematic plant cells or in animal cells. There the cytoplasm fills the entire cell, and vacuoles, if present, are small and scattered throughout the protoplast. The cytoplasmic mass in such cells is that of the volume of the entire cell minus the nucleus, and its measurement may be attempted with some degree of accuracy. In mature plant cells, however, any attempt to determine the volume of the cytoplasm proper would be inaccurate and of little value. For this reason the ratio of nucleus to the entire cell volume, rather than to that of the cytoplasm only, has been determined. This has been termed the cytonuclear ratio (Sinnott and Trombetta, 1936). If the vacuole is to be regarded as a part of the cytoplasm, this term would, of course, be synonymous with nucleo-cytoplasmic. In view of the sharp distinction between vacuole and cytoplasm in most mature plant cells, however, the term cytonuclear seems less ambiguous.

A criticism often made against studies of the volume relations of nucleus to cell is that it is hard to find material having nuclei and cells which may be measured accurately and also possessing a wide range in cell size. Measurement of cell volumes does present a difficult problem. A fairly close approximation to accuracy can be made, however, if the cells are spherical or of a simple geometric form like a cylinder. An even greater difficulty is often found in measurement of nuclear volumes. If the nuclei are spherical and suspended in the center of a cell, as they are in meristematic tissue, a fairly accurate determination of their volumes may be obtained; but if they are pressed against the wall, as in vacuolate cells, and thus become considerably flattened, the problem of obtaining volume measurements is a serious one. If these flattened nuclei are relatively symmetrical, dependable values may usually be obtained, but if they are irregular, an attempt to determine their volumes is practically impossible.

Because of these difficulties, a careful search was made for suitable material, having considerable range in cell size and fairly regular cells and nuclei. A variety of tissues was used, but chiefly stem tips and root tips of a number of representative families of the flowering plants, as well as leaves of *Elodea*, and stem hairs of the tomato. The following species were used:

Allium Cepa L.

Bryophyllum calycinum Salisb.

Cucumis sativus L.

Cucurbita Pepo L., inbred lines

Line-China

Line-Connecticut Field

Line—Connecticut Field  $\times$  China

Line-Mammoth Tours

Line—Pumpkin

Line-Straight Neck

Line-Spoon

Line-46

Elodea canadensis Michx.

Fagopyrum esculentum Gaertn.

Freesia refracta Klatt.

Glycine Soja Sieb. and Zucca (G. Max Merr.)

Helianthus annuus L. (Dwarf)

Helianthus annuus L. (Tall)

Hippeastrum puniceum Urban

Ipomoea tricolor Cav.

Lagenaria vulgaris Ser.

Lilium regale Wilson

Lycopersicon esculentum Mill.

Phaseolus multiflorus Willd.

Pisum sativum L.

Tropaeolum maius (Dwarf)

Tropaeolum maius (Tall)

Zea Mays L.

The seed of varieties of *Cucumis sativus*, *Cucurbita Pepo*, and *Lagenaria vulgaris* was supplied by Professor Sinnott.

In the case of leaves of Elodea and stem hairs of the tomato, living material was studied directly. The stem tips and root tips, however, were killed and sectioned. A number of different fixatives were tried, among them Flemming's, Navashin's, Modified Carnoy's, and Formalin-acetic-alcohol, but the most uniformly satisfactory results were obtained with "CRAF" (the chrom-acetic-formaldehyde formula of Randolph, 1935), with which little or no shrinkage was found to occur. All the material was treated in exactly the same manner so as to be strictly comparable. It was embedded in paraffin. Sections were made of the stem tips to a distance of approximately 5-15 millimeters behind the meristem; of the root tips, approximately 5 millimeters in most cases, but where a complete developmental history was desired, to a distance of approximately 70-90 millimeters. Heidenhain's iron-alum haematoxylin proved to be the best stain for such a study. Essentially similar results were obtained from a study of living and of preserved material.

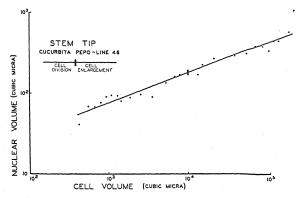


Fig. 1. Nuclear volume plotted against cell volume (both logarithmically) in cells of the stem tip of *Cucurbita Pepo* (Line 46).

Camera lucida drawings were made of the cells and nuclei; these drawings were measured and volumes calculated from them. A projected magnification of 1,700 was used. Meristematic cells of the root and stem tips are essentially isodiametric, and volumes were computed, from the average diameter, on the basis of a sphere. The results are thus probably somewhat too small but are comparable. The nuclei in such cells are spherical, and their volumes can thus be determined readily. Elongated, vacuolate cells of root and stem tips and of *Elodea* leaves are essentially cylindrical, and their volumes can be determined readily on this basis. In nuclei which have become somewhat flattened, if the polar diameter (a) and the equatorial diameter (b) are measured,

Table 1. Average cell and nuclear volumes in the meristem and region of enlargement of the stem of Cucurbita Pepo (Line 46).

Cell volume, cubic micra	Nuclear volume, cubic micra	Cytonuclear ratio (cell:nucleus)
426	41	10:1
553	69	8:1
680	68	10:1
820	77	10:1
950	92	10:1
1,100	95	12:1
1,305	94	14:1
1,440	81	18:1
1,889	90	21:1
2,580	98	26:1
3,600	91	39:1
5,350	137	39:1
6,830	164	42:1
8,000	169	47:1
13,450	172	78:1
15,050	228	66:1
21,400	271	79:1
38,850	291	133:1
53,400	311	172:1
67,500	385	175:1
82,400	383	215:1
98,500	349	282:1
128,400	456	282:1
174,800	599	292:1

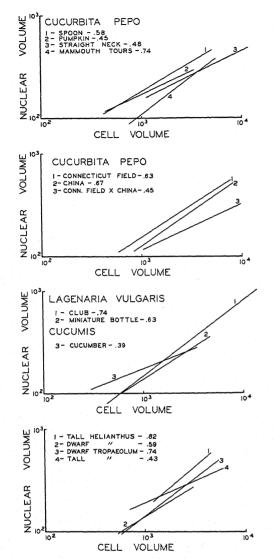


Fig. 2-5. Nuclear volume plotted against cell volume (both logarithmically) in cells of the root tips of various species. Measurements in cubic micra.

the volume may be computed by using the formula for a spheroid  $[4/3 \pi (a/2)^2, b/2]$ . If cells and nuclei as regular as possible in form are chosen, if measurements are made with care and in large numbers, and if the data are reasonably uniform and consistent, it is believed that the results obtained are dependable.

In an earlier paper (Sinnott and Trombetta, 1936), a method of analyzing the relationship between nucleus and cell was described similar to that proposed by Huxley (1932) for a study of heterogonic (allometric) growth. Here the logarithms of nuclear and of cell volumes are plotted against each other, the former on the y axis and the latter on the x axis. The graph in figure 1 illustrates this method. The points are from data given in table 1 in which nuclear and cell volumes for the growing point of

the stem of Line 46 of Cucurbita Pepo are shown. In this table the individual values for cell volume represent the average volume of successive cell-size classes, and the nuclear volumes represent the average nuclear values for the cells in this class (each average is one of 25–50 cells and nuclei). There is a considerable variability, doubtless due in part to difficulties of measurement, but the data fall into a rather narrow band to which a straight line may be fitted as shown in figure 1. Similar lines, without the points from which they have been determined, are shown in the other figures (fig. 2–13).

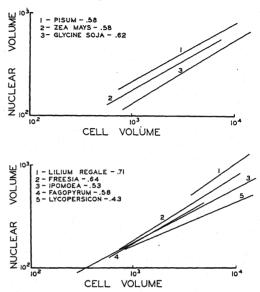


Fig. 6-7. Nuclear volume plotted against cell volume (both logarithmically) in cells of the root tips of various species. Measurements in cubic micra.

In the example used (table 1, fig. 1), the variation in cell size is great, from 426 to 174,800 cubic micra—a difference of 410 times. Nuclear variation is less, about 14 times. Evidently as cell size increases, nuclear size also increases but not as rapidly. In other words, with an increase in cell size, there is an increase in the cytonuclear ratio. That this is actually a case of growth of cells, not merely differences in cell size in the meristem itself, is indicated by the fact that only small cells are found in the active meristematic region. As one progresses back from the tip, the cells are increasingly larger. No uniform and constant relationship appears from a study of the table, but if the logarithms of nuclear and of cell volumes are plotted against each other, they fall along a straight line, indicating that the relative rate of change is constant and that a definite relationship can therefore be determined. This can be expressed by a constant derived from the slope of the line on the graph. Using the terminology of Huxley, x in this case is cell volume, y nuclear volume, b a constant denoting the value of y when x is unity, and k (or a in the terminology of Huxley and Teissier) the slope of the line, or the tangent of the

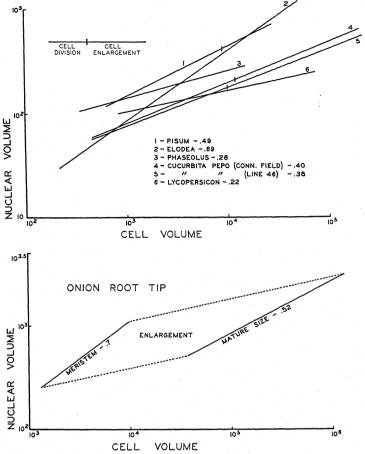


Fig. 8-9.—Fig. 8 (above). Nuclear volume plotted against cell volume (both logarithmically) in cells of the stem tips of various species. Measurements in cubic micra.—Fig. 9 (below). Nuclear volume plotted against cell volume in cells of the onion root tip.

angle which it makes with x. Thus  $y = bx^k$ , or log  $y = \log b + k \log x$ . The slope of this line in the present example, representing the value of the constant k, is about .38, which means that the rate of increase in nuclear volume (y) is about .38 as fast as that of cell volume (x). It is as though the nucleus were increasing at a compound interest rate of about 1 per cent and the cell at a rate of about 3 per cent. The arithmetic ratio between two such variables will evidently change progressively (as is shown in the table), since they are increasing at different rates. It is significant that it is the relationship between these rates, rather than any absolute values, which is constant. Even though the absolute cytonuclear ratio itself changes markedly, a definite relationship may therefore be determined between nuclear volume and cell volume. Such a method of analysis as employed here makes this relationship evident when a study of merely the absolute ratios does not do so.

RESULTS.—It was pointed out in the preliminary paper that these cytonuclear ratios are often specific and constant. In many cases the value of k approaches .67, indicating that the nucleus is growing about two-thirds as rapidly as the cell. Since the

surface of a cell increases as the square of its linear dimensions and its volume as the cube, and the surface thus increases two-thirds as fast as the volume, this result would indicate that the volume of the nucleus is keeping pace with the surface of the cell (or perhaps with the volume of cytoplasm in vacuolated plant cells). An examination of tables 2 and 3, and figures 1-13, however, shows that the value of k is often considerably above or below .67, although this value is near the average of all the determinations. In general, nuclear volume bears a definite relationship to cell volume, large cells having larger nuclei than small cells; but, as cell size increases, nuclear size does not increase as fast. A study of the data shows that there is a good deal of variation in the character of this cytonuclear ratio and suggests that no single explanation will suffice for all types. An analysis of these various types, however, should help to determine some of the factors involved.

Meristematic cells.—The root.—The k values obtained for root tip meristems of a number of plants are presented in table 2, and the graphs from which some typical ones have been determined are shown in figures 2–7. The values range from .39 in Cucu-

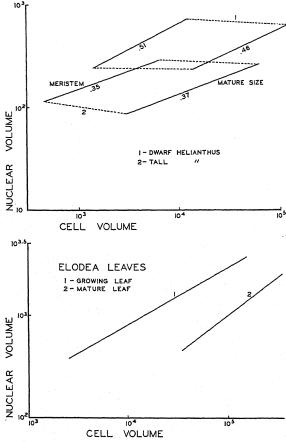


Fig. 10-11.—Fig. 10 (above). Nuclear volume plotted against cell volume (both logarithmically) in cells of stem tips.—Fig. 11 (below). Nuclear volume plotted against cell volume in cells of *Elodea* leaves. Measurements in cubic micra.

mis, to .82 in tall Helianthus, the average being .6. There are considerable and specific differences, how-

Table 2. The value of the relative growth constant k of nuclear volume to cell volume for meristematic cells of a number of different species.

of a number of any creat operator	
Root	
Cucumis	.39
Tropaeolum (Tall)	.43
Lycopersicon	.43
Cucurbita (Pumpkin)	.45
Cucurbita (Connecticut Field X China)	.45
Cucurbita (Straight Neck)	.48
Ipomoea	.53
Pisum	.58
Zea Mays	.58
Cucurbita (Spoon)	.58
Fagopyrum	.58
Helianthus (Dwarf)	.59
Glycine	.62
Lagenaria (Miniature Bottle)	.63
Cucurbita (Connecticut Field)	.63
Freesia	.64
Cucurbita (China)	.67
Amaryllis	.70
Allium	.70
Lilium	.71
Tropaeolum (Dwarf)	.74
Cucurbita (Mammoth Tours)	.74
Lagenaria (Club)	.74
Helianthus (Tall)	.82
Stem	
Tropaeolum (Tall)	.04
Tropacolum (Dwarf)	.09
Bryophyllum	.12
Lycopersicon	.22
Phaseolus	.28
Helianthus (Tall)	.35
Cucurbita (Line 46)	.38
Cucurbita (Connecticut Field)	.40
Pisum	.49
Helianthus (Dwarf)	.51
Elodea	.69
Inouea	.03

ever, and these are not constant for any family. Thus in the Cucurbitaceae, varieties of Cucurbita

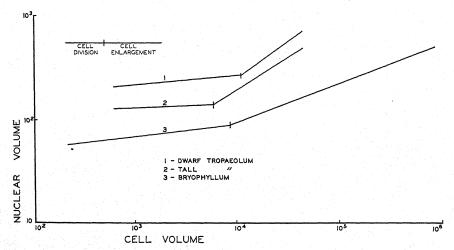


Fig. 12. Nuclear volume plotted against cell volume (both logarithmically) in cells of the stem tips of various species. Measurements in cubic micra.

Pepo range from .45 in Pumpkin, to .74 in Mammoth Tours (fig. 2, 3, 4). In all these, the cell increases faster than the nucleus, but the relative rates vary considerably. Similarly the value of k for tall Tropaeolum is .43, but for the dwarf .74; and for tall Helianthus it is .82, but for the dwarf it is much lower, .59 (fig. 5). Evidently plant height is not related to these differences. Other root meristems studied show similar variation (fig. 6, 7). Since the general average of these values is approximately .6, it is evident that the nucleus tends to grow about two-thirds as rapidly as the cell and that as a result

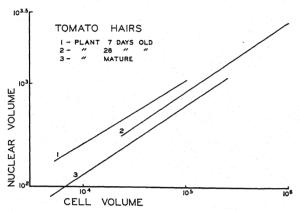


Fig. 13. Nuclear volume plotted against cell volume (both logarithmically) in cells of stem hairs of the tomato. Measurements in cubic micra.

the volume of the nucleus is keeping pace, in general, with the surface of the cell, though this relationship is subject to wide variability. The relationship in this tissue is essentially a true nucleo-cytoplasmic one, since meristematic cells are comparable to animal cells in being densely cytoplasmic, and any relationship of nucleus to cell is approximately one of nucleus to cytoplasm.

Significant differences appear in the level of the line in various species. This level (the value of b in the allometric formula) measures the difference in the absolute size relations of nucleus and cell at a given cell size. The cells of Pisum, for example, have relatively larger nuclei than cells of  $Zea\ Mays$ , or of  $Glycine\ Soja$  (fig. 6), though all the species have essentially the same k values.

The stem.—The k values obtained for stem tip meristems of a number of flowering plants are also presented in table 2, and the graphs constructed from the data are given in figures 8, 10, and 12. In nearly all the species, as in the root meristem, an increase in nuclear size accompanies an increase in cell size. The latter increases more rapidly, but the relative rates vary considerably, the values ranging from .69 in Elodea to .04 in tall Tropaeolum. Of significance in the stem meristem is the fact that the average value is considerably lower than that in the root. In three species (dwarf and tall Tropaeolum and Bryophyllum) the relative growth constants are very low (.09, .04, .12), showing that nuclear size

increases very much more slowly than cell size. In fact, there is little difference in the size of the nucleus in the entire meristem in these forms.

A comparison of the meristem cells in root and stem tips indicates that there is no marked difference in the absolute size of cell or nucleus in the two organs. In general, the roots have slightly larger cells and nuclei than the stems, but there are marked exceptions, such as dwarf *Helianthus*, where the nuclei and cells are larger in the stem.

Enlarging cells.—In view of the marked alterations undergone by plant cells as they pass from the meristematic condition to the much larger size and vacuolate condition at maturity, it is important to discover whether the cytonuclear ratio undergoes any change during this period. An attempt was made to determine this in several particularly favorable

types of material.

Three different phases of growth are generally recognized—the first, one of meristematic activity where cell division occurs; the second, one of enlargement, where there is a marked increase in size, accompanied by intake of water and development of a large vacuole; the third, one of differentiation, where the cell becomes mature. There are no sharp boundaries between these phases; cells in the upper region of the meristematic zone are undergoing enlargement, and cells undergoing enlargement have already begun to mature. This particular phase of the investigation is concerned mainly with the changes which take place during the phase of enlargement.

The difficulties encountered in such a study are serious. As the cell takes in a large quantity of water, a central vacuole is formed, filling most of it. This tends to flatten the nucleus (which until then has been suspended in the center of the cell) against the wall and makes it hard to measure accurately. Another difficulty is encountered in the preparation of unshrunken sections of root and stem tips in the region of cell enlargement. Several millimeters behind the tip, the cells are long, narrow, and very thinwalled. Fixation causes so much shrinkage and collapse of the walls that it proved impossible to obtain satisfactory material from which trustworthy measurements could be made in all types. Living material was more satisfactory at this stage and was studied in Elodea leaves and tomato hairs.

A study of this region of cell enlargement in a number of representative families of the flowering plants reveals three distinct types of cytonuclear behavior during development. In one type, the relationship of nucleus to cell found in the meristem is maintained throughout the active period of enlargement. Six such cases are shown in figure 8, and the k values for these species are presented in table 3. This has been found to occur in stem tips only. The approximate point at which the meristematic region ends and vacuolation begins was determined for only three of the six species, but from a study of the slides it is evident that meristematic activity ceases at approximately 10,000 cubic micra in all of them. The nucleus increases more slowly than the cell in all

Table 3. The value of the relative growth constant k of nuclear volume to cell volume for enlarging cells of a number of different species.

Root	
Allium	.52
Amaryllis	.52
Stem	
Lycopersicon	.22
Phaseolus	.28
Helianthus (Tall)	.37
Cucurbita (Line 46)	.38
Bryophyllum	.38
Cucurbita (Connecticut Field)	.40
Helianthus (Dwarf)	.46
Pisum	.49
Tropaeolum (Tall)	.63
Elodea	.69
Tropaeolum (Dwarf)	.72
Leaves of Elodea	
Growing leaf	.56
Mature leaf	.75
Tomato hairs	
7-day plant	.62
98 der plant	.67
28-day plant	.67
Mature plant	.07

cases, but the relative rates vary considerably. The significant feature is that the relationship of nucleus to cell found in the meristem persists throughout the period of vacuolation.

The second developmental type was found in root tips, stem tips, and leaves. Figures 9, 10, and 11 present typical examples. Here, the relationship of nucleus to cell which occurs in the meristem is not maintained throughout the period of enlargement. In the onion root tip (fig. 9), the meristematic phase of nuclear and cell growth is similar to that found in the other root tips described, the value of the relative growth constant being approximately .7. This relationship, however, is markedly altered during the period of active water absorption. Cell size increases enormously here, but nuclear size increases only slightly over that found in the meristem. When enlargement has ceased, a very similar size relation between cell and nucleus is again evident. In other words, during the period of active intake of water in the root, the k line in the meristem is shifted over markedly to the right and rises only a little. The change is mainly in the level of the line (the value of b in the formula). The slope of the line also changes slightly, for when the cells have reached their mature size, it is not so steep as in the meristem, the value of k being .52, indicating that the small cells of the meristem do not grow as much as the large ones. The fact that the k line in the meristem is shifted over progressively to the right suggests that for cells of different sizes but in the same stage of development, essentially the same cytonuclear relationship is probably maintained. In the development of an individual cell, however, from meristem size to maturity, the absolute ratio of nucleus to cell of course changes markedly. Cell size may increase as much as 100 times during the phase of water absorption; nuclear size increases only about 3 times at the most. A similar result was found in root tips of *Hippeastrum*.

It is assumed that as the k line shifts progressively to the right, during vacuolation, the relative sizes of cells remain constant, so that the smallest cells in the meristematic region are the smallest ones in the mature region. The question will naturally arise as to how one may be sure that such is the case since the history of individual cells was not followed. The subepidermal cell layer of the onion root tip offers good evidence on this point. This layer consists of alternating small and large cells which can be followed all the way back to the meristem. Each pair of cells arises by an unequal division in the meristem; by studying them, in succession, in a single row progressing back from the tip, one studies two cells which, although of different sizes, are of the same origin. Measurements show that even in the meristem the larger one has a larger nucleus than the smaller one, giving a k value of approximately .6 if a line is drawn through the two points. Successive measurements back from the tip indicate that the k line is shifted over progressively to the right and rises only a little, indicating a marked increase in cell size but only a slight one in nuclear size, precisely the situation postulated in the development of the root tip as a whole.

This same developmental type (the second) was also found in stems and leaves. Its occurrence in the stem is shown in figure 10. In dwarf Helianthus, as in the onion root tips, the k value in the meristem is .51, but in mature size the slope of the line decreases (.46), large cells growing more than small cells. That no nuclear size increase takes place during development is readily seen from the graph. Cell size increases considerably, but nuclear size remains the same as in the meristem. In tall Helianthus, nuclear size even drops a bit, although cell size increases enormously throughout development. A similar developmental history is found in Elodea leaves, where cell size increases considerably but nuclear size only slightly during enlargement (fig. 11). The slope of the line changes from .56 in the growing leaf to .75 in the mature one, indicating that the large cells do not grow as much as the small

The third developmental type was found only in stem tips of tall and dwarf Tropaeolum and in Bryo-phyllum (fig. 12). Here, as in the second type, the relationship of nucleus to cell found in the meristem fails to be maintained throughout the period of enlargement. As has already been pointed out for these three species, there is practically no nuclear growth in the meristem, the relative growth constants being .04 in tall Tropaeolum, .09 in dwarf, and .12 in Bryophyllum. Only when the end of mitotic activity is reached does nuclear size begin to increase, and from here on it shows a definite relationship with cell size. The constants are .63 for dwarf Tropaeolum, .72 tall, and .38 Bryophyllum.

These same general cytonuclear relationships are also manifest in a markedly different tissue, the stem hairs of the tomato (fig. 13). Multicellular hairs taken from the stem of a plant only 7 days old show a distinct relationship of nuclear and cell volumes, the relative growth constant being .62. The slope of the line is slightly different in hairs taken from a plant 28 days old and from a mature plant (.67 in each case). For three successive stages in plant development the cytonuclear ratio is thus essentially the same. A study of the level of the lines, however, shows a marked difference between the hairs of young and old plants, since in the latter the size of the nucleus for a given cell size is markedly lower. Hairs from a plant 28 days old are markedly smaller than those from a mature plant, indicating that the cells cut off by the apical meristem cell of the hair do not enlarge as much in the older plant. With maturity there appears to be a decrease in both cell size and nuclear size.

Discussion.—As a result of these studies on the cytonuclear ratio in plant cells, the conception of the relationship of nucleus to cell, as commonly found in the literature, evidently requires certain modifications. One major result of this investigation is very clear—the constancy which exists in the size relations of nucleus and cell is not between absolute values but rather between the relative rates of change of these structures during growth.

Thus the two conflicting views as to the cytonuclear relationship—that it is a constant and physiologically important one, or that it is extremely variable and therefore probably of little significance may be reconciled. Strasburger maintained that a constant size relation of nucleus to cell exists in the meristems of the higher plants, for he reports finding here a ratio of 2:3 between nuclear and cell diameters in a wide range of species. Obviously, however, such constancy as occurs cannot be one of absolute values, for the cytonuclear ratios are extremely variable when measured in absolute terms. It must rather be a constancy in relative rates of change. When volumes of meristematic nuclei and cells of a given species are compared, it is evident that there are marked differences in absolute cytonuclear values between large and small cells, but when they are plotted logarithmically, they fall along a straight line, showing a constancy of relationship. Large meristematic cells have relatively smaller nuclei in proportion to the size of the cell than small ones do. It is evident from such a study as this that the working-sphere of the nucleus is not a restricted one, but rather wide, as Bailey has pointed out.

The contrasted view of the cytonuclear ratio, as voiced by Bailey and others, regards it as an extremely variable one. This view is also open to criticism, for the variability is not random but follows a definite order with the result that a constancy does occur. Bailey found that the ratio of cell size to nuclear size in the elongated initials of the cambium of Pinus strobus varies greatly between large and small cells. If the method used in the present paper is applied, however, a relationship is found very

similar to that described here for meristematic cells. Although the cytonuclear ratio changes markedly in absolute terms, the relative rate of change is essentially constant. Similarly in studies of inanition in salamanders, Morgulis (1911) reports a wide variability in the ratio depending upon changes in the food supply. When the volumes of cells and nuclei reported by him are plotted logarithmically, however, the relative rate of change of nuclear and cell size is found to remain strikingly constant in spite of the fact that the absolute sizes of nuclei and cells decrease markedly during starvation and increase again when the animals are given food. Thus in many cases where the nucleo-cytoplasmic ratio has been reported as variable, a more precise analysis by the method here described will show that a constant relationship occurs, although it is between rates of change rather than absolute values.

The hypothesis of Boveri, Popoff, Hertwig, and others that a definite ratio of nucleus to cell is necessary before cell division will occur does not find support in these results. Mitosis occurs at very different ratios between these two variables in the same growing point, as a study of meristems of a number of species indicates. Extreme cytonuclear ratios, when the nucleus is either very large or very small in proportion to the size of the cell, may prevent mitosis, but in most meristems there is certainly no

constant ratio at which division occurs.

To find a physiological explanation for these definite cytonuclear relationships is a difficult task. As has already been pointed out (Sinnott and Trombetta, 1936), the frequency with which the relative size constant for nucleus to cell approaches .67 suggests that there may be a more definite relation than is apparent between volume of nucleus and of cytoplasm. If the thickness of the cytoplasmic layer remains constant in vacuolate cells of different size, then the area of the cell wall will be proportional to the volume of the cytoplasm which lines it. Since the surface of a sphere grows as the square of its linear dimensions and the volume as their cube, the surface of a spherical cell will increase two-thirds as fast as its volume; and since in many cells the volume of the nucleus has been shown to increase about two-thirds as fast as that of the cell, it is changing at the same rate as the surface of the cell. Thus if the volume of cytoplasm is proportional to the cell surface, the volume of the nucleus may be proportional to that of the cytoplasm, and there may actually be a constant nucleo-cytoplasmic ratio.

The marked differences in the way in which this ratio is manifest must also be explained. The changing relations of cell to nucleus which arise in so many species during the phase of cell vacuolation are perhaps an expression of differences in the water relations of these structures. The three developmental types which have been described above may possibly be explained by assuming differences between the nucleus and the rest of the cell in the absorption of water and dissolved materials. Differences in the ability of the cell to take in water by imbibition may be an important factor here, as may also differences in permeability between the nuclear and cytoplasmic membranes. Possibly a difference in osmotic concentration between cell sap and nuclear sap may be an important factor in regulating the amount of cell and nuclear enlargement. Variations in cell and nuclear growth in the meristematic region may also be due to variations in the rate of protein synthesis rather than of water absorption. These problems are important physiological ones but lie beyond the

scope of the present paper. The theory of senescence advanced by Minot appears to find some support from results here reported for tomato hairs. Between tomato plants 28 days old and mature ones, the size of cells in the multicellular hairs decreases considerably and of the nuclei even more, with a resulting decrease in the cytonuclear ratio. A mature plant is ordinarily less active metabolically than a young growing plant, as is indicated by the fact that assimilatory activity diminishes as a leaf ages (Willstätter and Stoll, 1918). There might well be a corresponding decrease in nuclear size with a decrease in metabolic activity. Some doubt is cast on this conclusion, however, by comparative measurements of nuclear volume in certain species. A direct indication of metabolic activity is sometimes provided by cell size itself, smaller cells being physiologically more active usually than larger ones, presumably because of their greater surface-volume ratio. Data from Pisum, Zea, and Glycine (fig. 6) show that the cell sizes for all three genera are essentially the same but that their nuclear sizes are different, Pisum being the largest, Zea next, and Glycine the smallest. If nuclear volume is a measure of metabolic activity, the three genera might be expected to differ in cell size as well.

As to whether changes in cell volume determine changes in the nucleus or vice versa cannot well be determined. In cases where nuclear volume is altered by changes in chromosome number, as in polyploidy, or in artificial nuclear doubling, cell volume follows the nuclear change. On the other hand, where the daughter cells from a given division are unlike, the nuclei, at first similar, soon accommodate themselves to the difference in cell volume. Nucleus and cytoplasm, of course, form a closely coordinated system, and it may be valueless to speculate as to which is the primary variable in development.

In view of the fact that the nucleus is the directive center of the cell and presumably controls its physiological, morphological, and genetical behavior, it seems highly important to discover how it exerts its influence. Most of the relations of nucleus

to cell are very difficult to discover, but the simpler quantitative ones, such as those reported here, can be measured with relative ease. A knowledge of these simpler relations is a first step toward an understanding of the more complex ones.

#### SUMMARY

The purpose of the investigation was twofold—to determine the quantitative relationship of nucleus to cell in meristematic plant cells and to show how this relationship is altered as these meristematic cells develop to their mature conditions.

Because of the difficulty involved in determining the nucleo-cytoplasmic ratio in mature, highly vacuolate plant cells, the relationship between nuclear volume and total cell volume (the cytonuclear ratio) was measured instead.

A variety of material was used, but chiefly stem tips and root tips of a number of representative families of the flowering plants, leaves of *Elodea*, and stem hairs of the tomato.

In most cases the nucleus increases in size less rapidly than the cell in cells of different sizes in the same stage of development, so that the actual cytonuclear ratio changes considerably. A constant relationship was found, however, between rates of change in size of the two structures, indicating a physiological interdependence between them.

In root meristems, the nuclear volume often increases about two-thirds as fast as the cell volume, thus keeping pace with the cell surface. This relationship is subject to wide variability, however. In stem meristems, the relative growth constants of nucleus to cell are lower than in the root.

In later development, where division has ceased but the cells are enlarging greatly, three distinct types of cytonuclear behavior were found. In one type, the relationship of nucleus to cell found in the meristem is maintained throughout the active period of enlargement. In the other two types, this relationship is greatly modified. Possible physiological explanations for these distinct types are suggested. The two conflicting views as to the cytonuclear relationship—that it is a constant and physiologically important one, or that it is an extremely variable one—may be reconciled by the results here reported.

A quantitative study of this type is the most direct method of approaching the important biological problem of the relation between nucleus and cell.

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# PHYSIOLOGICAL DIFFERENTIATION IN ASTRAGALUS WITH REFERENCE TO SELENIUM <sup>1</sup>

Sam F. Trelease and Helen M. Trelease

Some species of Astragalus appear to be definite selenium indicators, occurring only on seleniferous soils and always containing selenium when collected in the field (Beath, Eppson, and Gilbert, 1935, 1939a, 1939b). Other species of Astragalus, however, are not limited to seleniferous soils. Even when growing on such soils in proximity to selenium-accumulating species, these are free from selenium or contain very low concentrations. Since two of the selenium indicators—Astragalus racemosus and Astragalus pattersonii—were found to be greatly

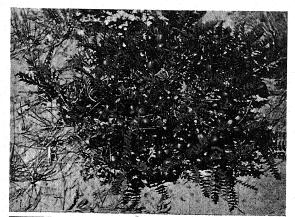




Fig. 1–2.—Fig. 1 (above). Astragalus crassicarpus Nutt. (prairie apple) growing on the Chugwater formation of the Triassic system about 7 miles east of Laramie, Wyoming. This species occurs on both seleniferous and non-seleniferous soils.—Fig. 2 (below). Astragalus racemosus Pursh growing on the Pierre formation of the Cretaceous system 15 miles south of Pierre, South Dakota. When air-dry, this specimen contained 3,920 ppm. of selenium. This species is a selenium indicator plant, apparently occurring only on seleniferous soils.

<sup>1</sup> Received for publication May 10, 1939. For general reviews of the selenium problem, see Trelease and Martin (1936), Beath (1937), and Moxon (1937). stimulated by selenium when cultivated in artificial media under controlled conditions, it was suggested that selenium may be an essential microtrophic element for the indicator plants (Trelease and Trelease, 1938).

In view of the apparent physiological differences within the genus Astragalus, it seemed of interest to study the influence of selenium on one of the non-indicator species when the plants were grown in solution and in sand cultures. For this purpose, Astragalus crassicarpus Nutt. was selected (fig. 1). One of the indicators, Astragalus racemosus Pursh (fig. 2), was included in the tests in order to secure comparable data on the two types of Astragalus.

MATERIALS AND METHODS.—Seeds of Astragalus crassicarpus, collected near Laramie, Wyoming, were kindly supplied by Prof. O. A. Beath; their selenium content, though undetermined, probably was low (Beath et al., 1935). The seeds of Astragalus racemosus were of the stock formerly used in culture studies; they were collected by us fifteen miles south of Pierre, South Dakota, and contained 2,125 ppm. of selenium. The culture methods were essentially the same as those previously employed (Trelease and Trelease, 1938). After being treated for twenty minutes with concentrated sulphuric acid, the seeds were soaked in tap water for three hours and then germinated in three-inch pots containing quartz sand. For the solution cultures, seedlings that were about three weeks old and 25 mm. high were removed from the sand and mounted in cork stoppers; each stopper bore five seedlings and fitted into a one-quart, jacketed Mason jar containing culture solution. During the early growth of the plants, the solutions were renewed every two weeks, and thereafter once a week.

For the sand cultures, ten seedlings were transplanted to three-gallon glazed earthenware jars plugged with coarse glass wool and filled with Ottawa silica sand ("flint shot"). The cultures were supplied with continuously renewed solution at the rate of one quart per day, by the method illustrated in figure 3 (Trelease and Thomson, 1935).

The plants were uniformly exposed to environmental conditions in the greenhouse. The period of illumination was extended by means of light from Mazda lamps (intensity about 250 foot-candles) from 5 to 10 p.m. The solution cultures of Astragalus racemosus were grown from December 4, 1938, to April 1, 1939. Those of Astragalus crassicarpus were also started on December 4 but were terminated earlier, on March 7, because of the withering and death of the plants in the highest concentration of selenium. The sand cultures grew from November 16, 1938, to April 7, 1939.

At the end of the growth period the plants in each jar were harvested and then weighed after having been dried for five hours in a forced-draft oven at 60°C. Analyses of samples for selenium were kindly made by Prof. O. A. Beath, H. F. Eppson, and C. S. Gilbert at the University of Wyoming. Some loss of selenium undoubtedly occurred during the drying of the plants, but this was considered to be unavoidable, since it was necessary to use dry weight as the criterion of growth.

All culture solutions contained the following concentrations of salts: .00072 m KH<sub>2</sub>PO<sub>4</sub>, .00048 m K<sub>2</sub>HPO<sub>4</sub>, .0040 m Ca(NO<sub>3</sub>)<sub>2</sub>, .0016 m MgSO<sub>4</sub>, .0016 m KNO<sub>3</sub>, .00053 m (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, .00004 m FeSO<sub>4</sub> (plus an equal concentration of K-citrate), and 15 cc. per liter of a stock solution (Trelease and Trelease, 1935) supplying the microtrophic elements Mn, B, Cu, Zn, Si, Al, I, Ni, Na, Li, As, and Co—of which only the first four are generally regarded as essential for higher plants.

Selenium was added as sodium selenite (Na<sub>2</sub> SeO<sub>3</sub>) in concentrations of 0.33, 1, 3, and 9 ppm. The S/Se ratio varied from 209 to 7.7 for the solutions containing from 0.33 to 9 ppm. of selenium, respectively; other ratios would have given somewhat different quantitative results (Trelease and Trelease, 1938). The initial pH value of the culture solutions was 6.4 (previously found favorable for Astragalus racemosus).

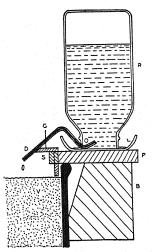


Fig. 3. Method of providing continuous renewal. Desired rate of flow is obtained by moving the apparatus to the right or left on the platform. After reservoir is refilled, the apparatus is reset by means of mark on tube D opposite guide nails G.

The conclusions drawn from this work are considered applicable only to the form of selenium used—selenite. The results might have been different with selenate (Hurd-Karrer, 1937) or with organic selenium obtained in water extracts of seleniferous plants (Beath, Eppson, and Gilbert, 1937).

GROWTH.—Table 1 and figures 4 and 5 show the influence of selenium (as selenite) on the dry yields of plants grown in solution cultures; table 2 gives the corresponding data for sand cultures.

The two species of Astragalus gave opposite responses to selenium. Astragalus racemosus was

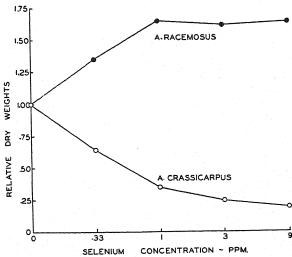


Fig. 4. Influence of selenium (as selenite) on the dry yields of plants grown in culture solutions. Astragalus racemosus is stimulated; Astragalus crassicarpus is poisoned.

greatly stimulated by selenium, whereas Astragalus crassicarpus was poisoned by it. In the sand cultures (table 2) the difference in the reaction of the two species was even more striking than in the solution

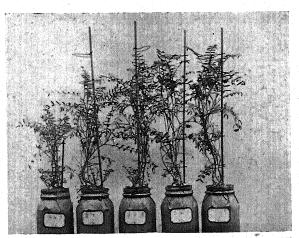




Fig. 5. Opposite effects of selenium (as selenite) on two species of Astragalus growing in solution cultures. Astragalus racemosus above; Astragalus crassicarpus below. Selenium concentrations in culture solutions (left to right): 0, 0.33, 1, 3, 9 ppm.

Table 1. Influence of selenium (as Na<sub>2</sub>SeO<sub>3</sub>) on dry yields of Astragalus racemosus and Astragalus crassicarpus grown in solution cultures.

Selenium concentration in culture solution, ppm.	Astragalus racemosus			Astragalus crassicarpus			
	Dry weight per culture of 5 plants		Selenium content of	Dry weight per culture of 5 plants		Selenium content of	
	Grams	Relative	plants, ppm.	Grams	Relative	plants, ppm.	
0	2.06			.62			
	1.43			.89			
	3.01			1.59			
	3.33			1.41			
	1.82			.80			
	2.33 ave.	1.00	29	1.06 ave.	1.00	8	
1/3 3	2.76			.99			
	3.58			.65			
	3.56			(.21)			
	2.63			.31			
	3.18			.78			
	3.14 ave.	1.35	99	.68 ave.	0.64	106	
	2.69			.43			
	4.93			.48			
	(1.41)			.27			
	3.45			.24			
	4.32			.45			
	3.85 ave.	1.65	234	.37 ave.	0.35	355	
	4.60			.24			
	2.66			.22			
	3.63			.22			
	3.95			.27			
	4.06			.31			
	3.78 ave.	1.62	355	.25 ave.	0.24	339	
	4.36			.20			
	4.07			.21			
	4.07			.15			
	2.95			.25			
	3.61			.21			
	3.81 ave.	1.64	726	.20 ave.	0.19	451	

Table 2. Influence of selenium (as Na<sub>2</sub>SeO<sub>3</sub>) on dry yields of Astragalus racemosus and Astragalus crassicarpus grown in sand cultures.

Culture solution	Astragalus racemosus			$A stragalus\ crassicar pus$			
	Dry weight culture of 10		Selenium content of plants, ppm.	Dry weight per culture of 10 plants		Selenium	
	Grams	Relative		Grams	Relative	content of plants, ppm.	
Minus selenium	10.4			10.97			
	14.8			8.35			
	5.7			9.63			
	7.7			5.78			
	8.6			7.45			
	9.4 ave.	1.00	12	8.44 ave.	1.00	4	
Plus seleniuma	29.5			.65			
	28.9			.87			
	25.9			1.24			
	23.8			1.01			
	23.9			1.88			
	26.4 ave.	2.81	1,090	1.13 ave.	0.13	141	

<sup>\*9</sup> ppm. Se for A. racemosus; 1 ppm. Se for A. crassicarpus (during first 12 days 9 ppm. was used).

cultures. The plants grew much more vigorously in the sand cultures, owing to better aeration of the roots and to continuous renewal of the culture solutions.

The results with Astragalus racemosus are in agreement with those previously reported for this species and for Astragalus pattersonii, and they suggest that selenium may be an essential microtrophic element for the development of various species of indicator plants.<sup>2</sup>

In being poisoned by relatively low concentrations of selenium (as selenite) in the culture solution, Astragalus crassicarpus resembles wheat, buckwheat, soy beans, tobacco, etc. (Martin, 1936; Hurd-Karrer, 1937; Martin and Trelease, 1938). Observations in the field have not yielded evidence for toxicity of selenium to this species, since neither native range plants nor crops have shown injury attributable to selenium in naturally occurring soils. These soils rarely contain more than about 10 ppm. of selenium, and much of this is in a form unavailable to any except selenium-indicator plants. Organic selenium, derived from native selenium-accumulating plants and often present in the soil, is much less toxic than inorganic selenite (Beath, Eppson, and Gilbert, 1937). We should therefore expect Astragalus crassicarpus to show a greater tolerance to organic selenium compounds than to the selenite used in the present tests.

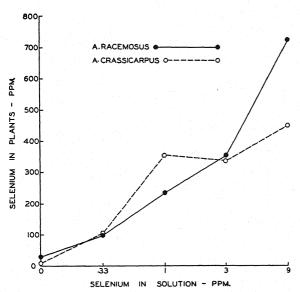


Fig. 6. Accumulation of selenium by Astragalus racemosus and by Astragalus crassicarpus growing in solution cultures.

Accumulation of selenium by the plants.—Analyses of the plants show no marked differences in the ability of the two species of *Astragalus* to absorb and accumulate selenium from solutions containing relatively low concentrations of sodium sele-

<sup>2</sup> It was not possible to deprive the controls entirely of selenium, since they received an initial supply from the seeds, which had a concentration of about 2,125 ppm.

nite (tables 1, 2, fig. 6). Astragalus racemosus, however, was able to accumulate considerably more selenium than Astragalus crassicarpus when the concentration in the solution was as high as 9 ppm. In an earlier study (Trelease and Trelease, 1938) it was found that Astragalus racemosus accumulated more than 3,000 ppm. of selenium when it grew in solutions containing 27 to 81 ppm. (as selenite)—concentrations that would be quickly lethal to Astragalus crassicarpus.

Although selenite is readily absorbed by Astragalus crassicarpus, as well as by wheat, buckwheat, tobacco, soy beans, etc. (Martin and Trelease, 1938), the pronounced susceptibility of these plants to selenium poisoning limits their accumulation of this element to a few hundred parts per million.

In contrast, the tolerance of Astragalus racemosus to selenium enables this species to accumulate very high concentrations. No symptoms of injury were observable when the selenium content was 726 ppm. (solution cultures) or 1,090 ppm. (sand cultures). In the study previously reported, marked reduction of growth did not occur until the selenium content of the plants exceeded 2,000 ppm. (Trelease and Trelease, 1938).

In the field, Astragalus racemosus shows no toxic symptoms even when it contains very high selenium concentrations. For example, the healthy plant shown in figure 2 contained 3,920 ppm. Well-developed specimens with a selenium content of 15,000 ppm. were obtained by Beath, Gilbert, and Eppson (1937). The high tolerance of plants in the field may depend upon the form of selenium present in the soil, since Beath and his co-workers have found that organic compounds of selenium, such as are obtained from water extracts of seleniferous plants, are more available and less toxic than the selenite used in our culture experiments.

Discussion.—The results of the present tests bring out a striking physiological differentiation within the genus Astragalus. This study, together with that previously reported (Trelease and Trelease, 1938), shows that Astragalus racemosus is greatly stimulated by selenium (as selenite) in the culture solution and possibly requires this element for its development. Astragalus crassicarpus, on the contrary, is not stimulated, but rather is poisoned by selenium in the nutrient solution—at least in concentrations of 0.33 ppm. (as selenite) or higher. The former species exhibits a high tolerance to selenium, withstanding a concentration of 81 ppm., but the latter is severely injured by 0.33 ppm. and is killed by 9 ppm.

These experiments seem to illustrate the physiological basis for the observations of Beath, Eppson, and Gilbert (1935, 1937, 1939a, 1939b) that Astragalus racemosus in the field is a selenium indicator, growing only on seleniferous soils and therefore useful for locating selenium areas. This species is distributed from North Dakota and Wyoming southward to Texas and New Mexico.

<sup>3</sup> The controls show low concentrations of selenium, possibly derived from the seeds.

Our culture experiments, showing that Astragalus crassicarpus is not benefited by selenium, are also in agreement with field observations that this species occurs on both non-seleniferous and seleniferous soils. It grows throughout the Great Plains area, from Manitoba and Montana to Texas and New Mexico, and in addition it extends eastward on to the non-seleniferous soils of Minnesota, Iowa, and Missouri.

In its physiological response to selenium, Astragalus racemosus appears to be like eighteen other indicator species of Astragalus and several species of Oonopsis, Xylorrhiza, and Stanleya (recently reported by Beath, Eppson, and Gilbert, 1939a, 1939b), whereas Astragalus crassicarpus seems to resemble alfalfa, wheat, buckwheat, tobacco, and soy beans, etc., as well as many other species of Astragalus, including A. carolinianus, A. diphysus, A. drummondii, A. flexuosus, A. missouriensis, etc.

Since the present experiments involved the use of sodium selenite (which all types of plants seem able to absorb), they failed to illustrate an important contrast brought out by the field studies of Beath, Gilbert, and Eppson (1935, 1937, 1939a, 1939b)—namely, that Astragalus racemosus is able to accumulate selenium from naturally occurring seleniferous soils, whereas Astragalus crassicarpus almost completely lacks this ability. These investigators have found that actively growing field plants of Astragalus racemosus always contain selenium.

The available analyses of Astragalus racemosus collected at different stages of development and on various types of soil are summarized in table 3

Table 3. Selenium contents of specimens of Astragalus racemosus collected at various stages of development and on various types of soils in the field (data from Moxon et al., 1938; Beath et al., 1937, 1939b).

	Specimens in each class				
Class, ppm. Se	Number	Percentage			
0	4,a	4.3ª			
0- 9	9ª	9.8ª			
10- 19	10ª	10.9			
20- 39	8	8.7			
40- 79	14	15.2			
80- 159	9	9.8			
160- 319	8	8.7			
320- 639	13	14.1			
640- 1,279	7	7.6			
1,280- 2,559	3	3.3			
2,560- 5,119	6	6.5			
15,000	1	1.1			

<sup>&</sup>lt;sup>a</sup> These specimens may have lost selenium in drying (see Beath et al., 1939a).

(data from Beath et al., 1939b, and Moxon et al., 1938). In considering the few non-seleniferous samples recorded in the table, it should be borne in mind that the specimens may lose selenium on maturing and especially on drying. Beath et al. (1939a) state that although some of their samples have given

negative tests for selenium, subsequent analysis of plants from the same soil area at a time when new growth was starting has invariably resulted in obtaining positive tests for selenium; they cite a case in which Astragalus bisulcatus (a selenium accumulator) in the past-seeding stage showed only 3.5 ppm. of selenium, while the following spring a composite sample of plants from the same area showed 135 ppm.

Table 3 illustrates wide variation in the selenium content of Astragalus racemosus collected in the field. In a number of cases the selenium content exceeded 1,000 ppm., and in one case it reached 15,000 ppm.

In the field, Astragalus crassicarpus, in contrast to the indicator species, is free from selenium or contains mere traces, even when growing on selenium-bearing soils (Beath et al., 1935, 1939a).

Astragalus racemosus, like other selenium accumulators, is poisonous to livestock on the ranges, but Astragalus crassicarpus is non-toxic and edible (as is indicated by its common names: prairie apple, ground plum, buffalo bean, and buffalo pea).

A further contrast, and one of great economic significance, is that Astragalus racemosus differs from Astragalus crassicarpus in being one of the selenium converters and soil contaminators that change selenium into organic, water-soluble forms which, when returned to the soil, are readily available for absorption by all types of plants, including grasses and common farm crops (Beath, Eppson, and Gilbert, 1935, 1937).

Physiological differentiation of plant species with reference to the absorption and utilization of selenium provides an entirely new approach to the study of plant relationships, and it may have an important bearing on the investigation of the taxonomy and evolution of the species now generally included in the genus Astragalus (or grouped, as by Rydberg, 1932, into a number of closely related genera). Response to selenium may be used as a taxonomic character in the classification of Astragalus species. Considerable progress in this direction has already been made by Beath, Gilbert, and Eppson (1939a, 1939b).

## SUMMARY

Astragalus racemosus, growing in solution and sand cultures, was greatly stimulated by selenium (as selenite) in concentrations of from 0.33 to 9 ppm.; these tests confirm earlier experiments in suggesting that selenium may be an essential microtrophic element for this species of Astragalus.

In contrast, Astragalus crassicarpus was not stimulated; it was instead poisoned by selenium, being severely injured by a concentration as low as 0.33 ppm. (as selenite).

Astragalus racemosus, having a higher tolerance than Astragalus crassicarpus to selenium, was able to accumulate correspondingly higher concentrations of this element from solutions containing selenite.

The greenhouse tests of growth in artificial media confirm field observations in showing a physiological differentiation of *Astragalus* species into two groups: those which seem to require selenium for their development and so serve as indicators of seleniferous soil areas, and those which do not utilize selenium.

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# THE GENERIC SEGREGATION OF THE SEQUOIAS <sup>1</sup>

#### J. T. Buchholz

The writer is thoroughly convinced that the Big Tree, Sequoia gigantea (Lindl.) Decn., and the Redwood, S. sempervirens (Lamb.) Endl., differ from each other to such an extent as to require their being segregated into separate genera. They differ by characters much more pronounced than those used to separate most genera within any of the families of conifers. That they should be looked upon as descendants of widely separated genera belonging to a larger group or family of world-wide distribution is abundantly evident from the study of paleobotany.

Throughout my investigations I had been under the impression that the results would lead to the recognition of Wellingtonia Lindl. (1853) as a valid generic name for the Big Tree, a name which has had some current use among European botanists. Wellingtonia, however, is a name applied by Meisner in 1840 to a dicotyledonous angiosperm, one of the Sabiaceae, in which usage it has been discarded, as a synonym, for the earlier Meliosma of Blume (1823). Wellingtonia Lindl. is therefore a later homonym, untenable as a generic name for the Big Tree, and to continue it in use is contrary to the current International Code of Botanical Nomenclature.

<sup>1</sup> Received for publication June 5, 1939.

Winslow considered the Big Tree a species of cypress, named it Taxodium Washingtonianum Winslow (1854), and expressed the wish that in the event that it should prove to belong to a distinct genus, it should be called Washingtonia californica. However, European botanists promptly referred the Big Tree to Sequoia Endl. where it has remained. Meanwhile, the name Washingtonia became established as a genus of palms, and it would be unfortunate to disturb it.

Other names under which the living Sequoias have been placed include Gigantabies, Senilis (1866) and Athrotaxis sensu Baillon (1892). The former was applied also to S. sempervirens, is a synonym of Sequoia Endl., and if considered equivalent to a generic name, was a superfluous name when published. Superfluous names have been rejected by the International Code of Botanical Nomenclature under Art. 16 and 60. Athrotaxis sensu Baillon is a broader concept which proposed the merger of the Sequoias with the Tasmanian genus Athrotaxis D. Don. The name Sequoia was first applied to the Redwood by Endlicher in 1847 and must remain as the generic name for this species. Thus it is clear that there is no generic name in the synonymy which

remains applicable to the Big Tree when segregated from Sequoia.

In the selection of an appropriate name for the Big Tree, it is very desirable that no word be selected which is wholly foreign to the names long in use. The name Sequoiadendron, which provides adequately for necessary generic distinction, does not wholly discard the long established name, Sequoia, and has obvious advantages in catalogues and indices. Furthermore, neither the resulting new binomial nor the continued use of the old botanical name will cause serious confusion.

While the Latin description given below is confined to the external diagnostic features which serve to separate the new genus Sequoiadendron from Sequoia, it seems desirable here to catalogue in a concise form all the more important external differences, some of which would have value only in distinguishing two species. While nearly any pair of these characters will serve to separate these two monotypic genera artificially, those in italics have generic significance. To these about 16 other histological and embryological differences are added, mostly of generic value and described elsewhere (Buchholz, 1938, 1939a, 1939b). These weigh heavily in confirming the conclusion that we are concerned here with two distinct genera. The most important generic differences are as follows:

Seed cones maturing and shedding seeds within year; ovuliferous scales becoming obliquely peltate with ovules and seeds attached in a single row near margin; buds scaly, foliage dimorphic in part taxoid with large leaves petioled, cotyledons usually 2. Sequoia Endlicher desc. emend.

Seed cones requiring two seasons to mature embryos and seeds and remaining alive and green for many years after maturity of seeds; ovuliferous scales becoming wedge-shaped, bearing two series of ovules and seeds; buds naked, foliage juniperoid, cotyledons usually 4. Sequoiadendron.

Sequoiadendron gen. nov.—Strobili polleniferi solitarii sessiles, in extremis ramulorum inter ramulos minimos lati. Strobili feminei plerumque in ramulis magnis subducentibus proximis ramulis ducentibus ramorum lateralium lati, squamae illorum 7–12 vel etiam plura ovula nuda erecta ferentes in serie duplici crescentiformi prope earum bases, eae spinis longis teretibus terminatae; ovula mox invertenda dum strobilus et ovula increscunt multiplicabilia strobilus ad 5–7 cm. longitudine crescit et biennium ut embryones maturescant requiritur. Strobili seminiferi virides manent et multos annos post maturitatem seminum persistunt. Embryones cum 3–5 cotyledonibus.

Arbor strobilifera gigantea cum gemmis nudis. Ramuli vestiti foliis parvis simplicibus imbricatis subulatis appressis vel non numquam divergentibus et spiraliter dispositis, bases rhomboides horum juvenem caulem tegentes; omnia folia unius speciei neque cum petiolis neque disticha divergentia.

Synonyms:

Condylocarpus Salisb. ex Lambert Gen. Pinus, quarto ed. 120 (1832), as synon.; not Desf. (1822).

Steinhauera Presl. in Sternberg, Fl. Forwelt, 2: 202 (1838), nom. rejiciend.

Sequoia Endlicher, Syn. Conifer. 197 (1847) in part.

Wellingtonia Lindley, Gard. Chron. 1853: 819-823; not Wellingtonia Meisner (1840).

Gigantables [Nelson] Senilis, Pinaceae 77 (1866) in part.

Sequoia Sargent, Silva of North America 10: 139 (1896) in part.

Athrotaxis sensu Baillon, Hist. Pl. 12:39 (1892) in part; not Athrotaxis D. Don (by error as G. Don), Trans. Linn. Soc. Lond. 18:171 (1839).

The type of the genus is the species:

Sequoiadendron giganteum (Lindl.) comb. nov., descr. emend.

Synonyms:

Wellingtonia gigantea Lindley, Gard. Chron. 1853: 819-820, 823; Hooker's Journ. Bot. & Kew Gard. Misc. 7: 26 (1855).

Taxodium Washingtonianum Winslow, Calif. Farmer (?Sept.) 1854; ex Hooker's Journ. Bot. Kew Misc. 7: 29 (Jan. 1, 1855), nom. illegit.3

Washingtonia californica Winslow, Calif. Farmer (?Sept.) 1854; ex Hooker's Journ. Bot. Kew Misc. 7: 29 (Jan. 1, 1855), nom. illegit.<sup>3</sup>

Sequoia gigantea Decaisne, Bull. Soc. Bot. France 1: 70(?Aug. 1854); Rev. Hort. Jan. 1, 1855: 9, fig. 1; notSequoia gigantea Endlicher Syn. Conif. 198 (1847).

Sequoia Wellingtonia Seemann, Bonplandia 3: 27 (Feb. 1, 1855).

Taxodium giganteum Kellogg & Behr, Proc. Calif. Acad. Sci. 1: 53 (1855).

Gigantabies Wellingtoniana [Nelson] Senilis<sup>2</sup> Pinaceae 79 (1866).

Sequoia Washingtoniana (Winslow) Sudworth, Bull. U. S. Forest Service No. 14: 61 (1897) including 8 varieties fide Sudworth.

Steinhauera gigantea Ktze. ex Voss in Mitt. Deutsch. Dendrol. Ges. 16: 90 (1907).

Range: Western slope of the Sierra Nevada Mountains of California, associated with Sugar Pine, Fir, and Incense Cedar, in the California Arid Transition Zone, from the southern boundary of Placer County to the head of Deer Creek in Tulare County. Distributed in groves, widely separated in the north, but forming a series in a large nearly continuous area in the basins of the Kings, Kern and Tule Rivers, at altitudes between 4,500 and 5,500 feet in the north and between 5,500 and 6,500 feet or more in the south.

Type locality: Calaveras Grove, Calaveras County.

The external taxonomic characters are fully exemplified by specimens showing pollen cones, ovulate cones before pollination, and seed cones of previous years collected by the writer during April, 1936, at General Grant

<sup>2</sup> Nelson (John), under the pseudonym Senilis, wrote a book on Pinaceae (1866). He was an arboriculturist, living at Rinefield, Lymington, Hants. He renamed many of the conifers, following a system which he described as non-Linnean, according to which his binary names were compounded from categories much higher than a genus where there are not many forms in a sub-division, and from lower categories in groups embracing many species. His name "Gigantabies" is not a genus but in the higher category as a sub-division. This name applied equally to the two Sequoias, was a superfluous name when published, and has been declared illegitimate by the International Rules of Botanical Nomenclature (Art. 16 and 60). See also Jackson, B. D., p. 140, Guide to Lit. of Bot. 1881.

<sup>3</sup> The names given by Winslow are illegitimate names since they are nomina alternativa or eventualia (see Int. Rules of Bot. Nomenclature, 1935).

National Park. These have been deposited in the Herbarium of the University of Illinois, also similar specimens at Stanford University, the University of California, and elsewhere.

Summary of outstanding generic and specific differences

Sequoiadendron giganteum

Pollen cones 4-6 mm. long terminally sessile and solitary on small twigs.

Ovulate cones terminal on larger twigs resembling leaders of lateral branches, scales 25-40.

Cone scales bearing at pollination 3-12 or more erect ovules in double crescentic row.

Cone scales terminated by long terete spine, somewhat persistent.

Ovules becoming inverted during enlargement, to form single or double row of 3-9 seeds on surface of wedge-shaped scales.

Seed cone becoming 5-7 cm. long, cone axis very stout and woody; scales not easily broken off.

Seeds requiring two seasons for maturity of embryos.

Seed cone remaining green and attached to tree for many years after maturity of seeds.

Seeds 5-7 mm. long with 2 thin wings broader than body of seed.

Seeds yield ±200 per cone. Buds naked.

No vegetative reproduction.

Leaves all small, of only one kind, spirally imbricated, appressed or sometimes spreading, not petioled, revolute or distinctly ribbed, persisting and clothing the 2-5-year-old stem with rhomboidal areas.

Wood light (Sp. gr. 0.29) brittle, tending to fracture transversely. Sequoia sempervirens

Pollen cones stipitate.

Ovulate cones terminal on larger twigs, clothed with scale leaves. Scales 15-20.

Cone scale bearing at pollination 3-7 erect ovules in single arched row.

Cone scales terminated by long flattened spine, usually deciduous.

Ovules becoming inverted during enlargement, to form single row of 2-5 seeds near margin of obliquely shield-shaped scales.

Seed cone becoming 2-3 cm. long, cone axis relatively slender; scales of dry cone easily broken off.

Seeds requiring only one season for maturity of embryos.

Seed cone turning brown and shedding seeds at maturity.

Seeds 3-4.5 mm. long with 2 spongy wings not as broad as body of seed.

Seeds yield  $\pm 60$  per cone. Buds scaly.

Abundant vegetative reproduction.

Leaves dimorphic, the vigorous terminal shoots having small scale-like leaves, other branches bearing large scytheshaped, petioled leaves acute with prominent midrib and revolute margins, pale beneath and spreading in two ranks decurrent and clothing the 2-4-year-old stem with elongated cuneate areas.

Wood heavier (Sp. gr. 0.42) tough, tending to fracture lengthwise only.

Bark becoming 25-50 cm. thick, deeply furrowed or fluted in lobes up to 1 m. or more wide, dull reddish brown, tinged with purple on young trees.

Stem habit—becoming stout, branches turning upward at tip.

Becoming largest tree known.

Bark becoming 15-25 cm. thick, in ridges corresponding to ridges in trunk, up to 50 cm. or more wide; the bark furrowed with smaller ridges into long, narrow, fibrous scales often broken transversely, exposing a rich cinnamon red bark.

Stem habit—remaining more slender with horizontal or drooping branches.

Becoming tallest tree known.

There are several other differences, such as time of pollination—April–May vs. January–February—time of seed cone enlargement and of fertilization; differences which are also very marked where the two species are growing side by side in cultivation. The pollen grains of the Big Tree are  $23 \mu$  in diameter ( $\sigma = 1.5$ ), and those of the Redwood are  $33 \mu$  in diameter ( $\sigma = 2.6$ ) when expanded.

## Internal gametophytic differences

Sequoiadendron giganteum

Male gametophyte long and slender. Enlarged only at fertilization tube.

Female gametophyte alveolar in development throughout, similar to other conifers.

Usual condition of only one female gametophyte per ovule.

Relatively few (±20) archegonia, not all fully developed.

Jacket cells of archegonia none or relatively inconspicuous.

Megaspore membrane relatively thick, 2-3\(\frac{1}{2}\)\(\mu\).

Chromosomes—n = 11.

Sequoia sempervirens

Male gametophyte shorter, stouter. Widest at middle, sometimes branched or saccate.

Female gametophyte with free nuclei scattered throughout cytoplasm at both ends; alveolar only in central part.

Several female gametophytes per ovule in competition during development.

Very many (50-200) differentiated structures appearing as if archegonia, not all fully developed.

Abnormally large and irregularly scattered jacket cells.

Megaspore membrane relatively thin, less than 1  $\mu$ .

Chromosomes—n = 22.

#### Embryological differences

Sequoiadendron giganteum

Proembryo with free-nuclear stage.

Proembryo or ganized somewhat in tiers.

Prosuspensor present in embryo system.

Rosette cells and rosette embryos.

Sequoia sempervirens

Proembryo has no free nuclei.

Proembryo not organized in tiers.

No prosuspensor in embryo system.

No rosette embryos.

Cleavage polyembryony gives 10-12 or more embryos.

Cell volume average small.

Suspensor segments becoming very long and

slender.

Embryonal tubes successive in formation, replacing primary suspensors.

Embryo has 3, 4, or 5 cotyledons (mean = 3.7). Cleavage polyembryony usually gives 4 embryos.

Cell volume average large. Suspensor segments not so long. Much more stout.

Embryonal tubes nearly simultaneous and surrounding primary suspensor.

Embryo usually has 2 cotyledons.

There has been considerable discussion and some confusion as to the proper specific epithet for the Big Tree within the genus Sequoia. My selection of

Lindley's species name, giganteum, is based on unquestionable priority, and in combination with Sequoiadendron its use is legitimate.

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## NOTES ON SOME MIDDLEWESTERN SPECIES OF BAPTISIA 1

## Mary Maxine Larisey

Baptisia australis (L.) R. Br. has for many years been the classical "dumping ground" for material collected in the middle west. There can be little doubt as to the true nature of B. australis, because for no other species is there such a wealth of literature, copiously illustrated. As originally described, it is a beautiful blue-flowered, large-leaved, simpleand erect-branched species of the eastern states and has been widely cultivated.

Throughout the middle west a blue-flowered. smaller-leaved, dichotomously and divaricately branched plant occurs in great abundance, and for many years this smaller type has been taken for B. australis. A comparison with authentic B. australis makes it very clear that the more western type is specifically distinct. Discovery of the type specimen of B. minor Lehm. in the Gray Herbarium indicates that this fact was recognized as early as 1827. In 1861 it was again described as B. texana by Buckley. But neither Lehmann's nor Buckley's name has ever received much attention. Not until 1932 when Small in Rydberg's "Flora of the Prairies and Plains of Central North America" described B. vespertina from Missouri, Kansas, and Texas was any widespread cognizance of its existence manifested. But Small's name, which has been recognized in the last few years, must go into synonomy along with B. texana. The true B. australis is confined to the area within Pennsylvania to southern Indiana, south to Virginia and Tennessee. It has been introduced in Vermont.

A list of the essential differences between the two species in question follows:

B. australis

B. minor

Plant 1.5 m. high.

Plant 0.6-1.2 m. high.

Stem stout, branches ascending.

Stem slender, branches divaricate.

<sup>1</sup> Received for publication June 15, 1939.

Leaflets obovate-lanceolate, 4-8 cm. long, 1.5-3 cm. broad, delicate.

Racemes 2-5 dm. long.

Mature pod oblong, slightly inflated, 3.5-4 cm. long, 1-1.5 cm. broad, abruptly beaked, stipe not exceeding the calyx.

Distributed along riverbanks, eastern states.

Leaflets obovate, 2-3.4 cm. long, 0.5-1 cm. broad, firm.

Racemes 1-2.5 (rarely 3.5) dm. long.

Mature pod oblong-elliptic, much inflated, 3.5-6 cm. long, 1.5-2.5 cm. broad, gradually beaked, stipe twice as long as the calyx.

Distributed on dry hills and prairies, middle western states.

A bibliographical treatment and description of *B. minor* follows:

B. minor Lehm. Ind. Semin. Hort. Hamb. 16. 1827; Linnaea 3: Litt. 119. 1828; Nov. Act. Nat. Cur. (Leop. Carol. Deutsch. Akad. Naturforsch.) Nova Acta 14: 803. 1828 (1829).

B. australis (L.) R. Br. var. β, Torr. & Gray, Fl. N. Am. 1: 385. 1840.

B. texana Buckl. in Proc. Acad. Nat. Sci. Phila. 13: 452. 1861; Proc. Acad. Nat. Sci. Phila. 14. 163. 1862.

B. vespertina Small ex Rydberg, Fl. Prairies & Plains Central N. Am. 456. 1932.

B. australis (L.) R. Br. var. minor Fernald, Rhodora 39: 312. 1937.

Plant 6-12 dm. high, glabrous throughout; stem slender, geniculate, widely dichotomously branched; leaves subsessile to short-petiolate, petioles 2-4 mm. long, leaflets obovate to obovate-cuneate, occasionally elliptic, apiculate, rarely obtuse or retuse, 2-3.4 cm. long, 0.5-1 cm. broad; stipules lanceolate to setaceous, 3-9 mm. long, persistent or caducous; racemes terminal, erect, compact; floral bracts cordate to ovate-lanceolate, 7-9 mm. long, caducous; pedicels stout, 5-7 mm. long; calyx-tube 9-10 mm. long, 6-8 mm. broad, upper lip subconnate, lobes of lower lip usually ovate, sometimes acute, 2-3 mm. long; corolla dull violet to deep blue, inner petals lighter, fre-

quently tinged with yellow, standard 2.5 cm. long, wings and keel 2.7–3 cm. long; mature pod oblong-elliptic, ligneous, much inflated, 3.5–6 cm. long, 1.5–2.5 cm. broad, tapering gradually into a short beak, long-stipitate, stipe twice as long as the calyx.

Distribution: dry hills, limestone glades and prairies, Missouri and Kansas, southwest to Texas; introduced

along railroads in Nebraska.

Citation of type and authentic specimens:

Kansas: along the road 4 mi. E. of South Haven, July 4, 1929, Rydberg & Imler 579 (NYB, authentic specimen of B. vespertina Small); camp ground, vicinity of Coldwater, July 6, 1929, Rydberg & Imler 676 (NYB, authentic specimen of B. vespertina Small).

Texas: northern Texas, May, Buckley (ANSP, TYPE

of B. texana Buckley).

Cultivated specimen: ex Hort. Hamb. "A." 1827, Lehmann (GH, TYPE).

The relatively frequent occurrence of hybridism, particularly in regions where the borders of the geographical ranges of some of the larger species overlap, has been the basis of much confusion within the genus. One of the most clearcut cases is that between B. minor Lehm. and B. leucophaea Nutt. in southwestern Missouri, Kansas, and Oklahoma. In general habit it more nearly resembles B. minor, but the flowers are bicolorous: the standard is blue as in B. minor, the wings and keel yellow as in B. leucophaea. Back-crosses to B. leucophaea are apparently common. Since this hybrid recurs in a number of localities in the vicinity of both parents with a fair degree of intermediacy between them, it seems advisable to propose a binomial and description:

 $\times$  B. bicolor Greenman & Larisey, hyb. nov.<sup>2</sup>

B. minor  $\times$  B. leucophaea.

Intermediate between B. minor and B. leucophaea: Plant less than 1 m. high; leaflets oblanceolate-spatulate or broadly obovate; racemes axillary, frequently flexuous, usually not secund; corolla bicolorous, standard purple, wings and keel cream or yellow.

Distribution: prairies, southwestern Missouri, Kansas,

and Oklahoma.

Citation of specimens:

Missouri: frequent on upland prairies, Webb City, Jasper Co., May 4, 1902, Palmer 127 (MBG, TYPE); rocky upland prairies near Webb City, Jasper Co., May 10, 1931, Palmer 39282 (MBG, GH, NYB).

Kansas: rich, black soil, stony prairie land 1 mi. S. W. of College, Riley Co., May 7, Bayliss (KA); about 2.5 mi. S. W. of Iron Mound, Saline Co., May 3, 1930, Hancin 215 (KA); hilltop near Manhattan, Riley Co., May 21,

1920, Herr 9 (KA).

Oklahoma: prairie near Tecumseh, April 22, 1932, Barkley 47 (MBG, UO); prairie 2 mi. E. of Norman, May 10, 1924, Bruner (USN); Fort Sill, Comanche Co., April 17, 1916, Clemens 11623 (GH); 6 mi. N. W. of Stillwater, May 18, 1931, Featherly (MBG); Ripley, July 17, 1931, Featherly (MBG); in open woodlands, S. Okla., near Interstate bridge, April 11, 1928, Nelson 10816 (MBG).

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<sup>2</sup> Inter minorem et leucophaeam media; herba vix 1 m. alta; foliola oblanceolato-spatulata vel anguste obovata; racemi axillares frequenter flexuosi plerumque non secundi; corolla bicolorata, vexillo purpureo, alis carinaque flavis vel gelvis.

# DEVELOPMENT OF THE EMBRYO SAC OF PLUMBAGELLA MICRANTHA 1

J. W. Boyes

Plumbagella Micrantha (Ledeb.) Spach is a small herbaceous annual, the only member of its genus, which grows in the mountains of Siberia. It is known also under the synonyms *Plumbago micrantha* Ledeb. and *Plumbaginella micrantha* Ledeb.

Since the extensive studies of Dahlgren (1915, 1916) on the Plumbaginaceae, interest in Plumbagella has grown. His work centered interest upon the method of embryo-sac development which he described for members of the sub-family Plumbagineae. The species studied included Ceratostigma plumbaginoides Bunge, Plumbago capensis Thunb., P. pulchella Boiss., P. zeylanica L. and Plumbagella micrantha (Ledeb.) Spach. The last-named species was the one most thoroughly investigated.

Dahlgren considered the development of the embryo sac in *Plumbagella micrantha* as much reduced. The macrospore mother cell functions as an embryosac mother cell. After the first meiotic division, a vacuole separates the two resulting nuclei. This division is followed only by the homoeotypic division, on completion of which the embryo sac is four-nucleate. The vacuole just mentioned, now somewhat

<sup>1</sup> Received for publication May 18, 1939.

enlarged, separates the two nuclei nearer one end of the embryo sac from the two nearer the other end. The nucleus nearest the chalazal end becomes the nucleus of the single antipodal cell; that nearest the micropyle becomes the nucleus of the egg; and the other two nuclei unite to form a diploid primary endosperm nucleus. Since only the two meiotic divisions seemed to be involved, this method of embryosac formation was of particular interest.

Haupt (1934) reinvestigated the development of the embryo sac in *Plumbago capensis* and agrees with Dahlgren's story for the same species up to the four-nucleate stage. The four nuclei, however, divide, and the daughter nuclei remain in pairs, one pair at each end and one pair at each side of the sac. One micropylar nucleus becomes the egg nucleus. Four nuclei, one from each pair, migrate to the center, where they fuse to form a tetraploid primary endosperm nucleus.

Dahlgren (1937) later concluded that embryo-sac development in *Plumbago zeylanica* and *Ceratostigma plumbaginoides* follows the sequence reported by Haupt for *Plumbago capensis*. He still considered, however, that his investigation of *Plumbagella* 

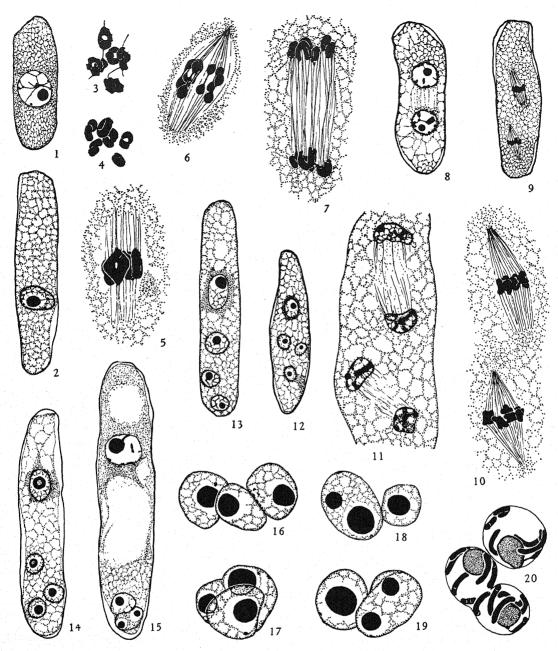


Fig. 1-20. All figures were drawn with the aid of a camera lucida. The micropylar end in these and later figures is in each case upward.—Fig. 1. Embryo-sac mother cell.—Fig. 2. Unusually large embryo-sac mother cell.—Fig. 3. Prophases of the heterotypic division, lateral view.—Fig. 4. Equatorial plate, heterotypic division, polar view (n=6).—Fig. 5. Equatorial plate, heterotypic division, polar view.—Fig. 6. Metaphase, heterotypic division, lateral view.—Fig. 8. Two-nucleate interkinetic stage.—Fig. 9. Embryo sac showing lateral views of the homoeotypic divisions.—Fig. 10. The same homeotypic figures as in fig. 9, enlarged.—Fig. 11. Organization of four nuclei at the end of the homoeotypic division.—Fig. 12. Young primary four-nucleate embryo sac.—Fig. 13. Slightly older primary four-nucleate embryo sac, showing nearly linear arrangement.—Fig. 14. Migration of three nuclei to the chalazal end.—Fig. 15. Embryo sac showing two nuclei united and a third very close, at the chalazal end.—Fig. 16. Three closely associated chalazal nuclei.—Fig. 17-19. Cases of the union of two chalazal nuclei.—Fig. 20. Chalazal nuclei in the prophases of the third division after two nuclei have partly united. Fig. 1, 2, 8, 9, 12-15, are × ca. 920; all others × ca. 2330.

was so thorough that development in that species cannot be the same as in *Plumbago*.

Fagerlind (1938a) has still more recently studied the development of the embryo sac of *Plumbagella*. The history outlined by him resembles, save in certain minor points, the usual method of development as described in the present paper. It is very different from that described by Dahlgren for this species.

My results have been briefly reported in a pre-

vious paper (Boyes, 1939).

This study has been conducted during the tenure of a Wisconsin Alumni Research Foundation Scholarship. The writer wishes to express his sincere thanks for the helpful criticism and kindly advice, given freely throughout the course of the investigation, by Dr. C. E. Allen.

MATERIALS AND METHODS.—With the help of Dr. George M. Reed of the Brooklyn Botanic Garden, to whom thanks are due, fruits of *Plumbagella micrantha* were obtained from the directors of the Botanical Gardens of Marburg, Copenhagen, Leyden, Bremen, and Palermo.

Seedlings were obtained from the fruits from Copenhagen and Leyden. Mature plants from the two sources were similar except that the stems of the Leyden plants were slightly reddish, whereas

those of Copenhagen plants were green.

The mature plants varied greatly in size at maturity, apparently being strongly influenced by environmental conditions prevailing in the greenhouse during the growth period. Those grown during the winter reached a height of only three or four inches in ordinary daylight. When artificial light was supplied at night, the plants grew to a height of a foot or slightly more but were of less erect habit. During July and August in ordinary daylight they reached a height of nine or ten inches.

Buds were cut from the inflorescences immediately before fixation. Pistils were removed for fixation from buds longer than about 2 mm.; smaller

buds were fixed entire.

Randolph's and Belling's modifications of the Navashin fixative were used most extensively for fixation. Both gave better results if the material was first dipped for 5–10 seconds in a 6:3:1 Carnoy solution. Fixation was aided also when materials in the fixative (whether or not prefixed in Carnoy's solution) were placed for a few minutes under a vacuum pump. Fixation in any of the Carnoy solutions (6:3:1, 3:1, and 2:1) alone was not consistently satisfactory. The Randolph modification in combination with prefixation with or without pumping treatment seemed superior to the Belling modification and was therefore chiefly used.

All material was dehydrated in alcohol, cleared in chloroform, imbedded in paraffin, sectioned at 15-20 microns, and stained in Heidenhain's ironalum haematoxylin. A few buds were stained with

Flemming's triple stain.

OBSERVATIONS.—As Dahlgren (1916) has reported, a single subepidermal archesporial cell gives rise to a parietal cell and a macrospore mother cell,

the latter of which functions as an embryo-sac mother cell.

The usual history.—As the nucleus of the macrospore mother cell prepares for the first meiotic division (fig. 1), the cell elongates and increases slightly in diameter. This nucleus is still in the early heterotypic prophases at the time that the meiotic divisions are completed in the microspore mother cells. The mother cell continues to enlarge slowly during the prophases until its relative size is ordinarily approximately that shown (after the completion of the heterotypic division) in figure 8. Very rarely, while still uninucleate, it becomes as large as or even larger than that shown in figure 2. Six chromosome pairs were clearly counted in the later prophases (fig. 3) and in the heterotypic equatorial plate (fig. 4). Usually the spindle (fig. 5, 7) lies near the center of the cell and parallel to its longer axis, but it may lie diagonally (fig. 6). Thus the two resulting nuclei, which pass into a brief interkinesis (fig. 8), commonly but not always lie in the long axis of the cell.

The two-nucleate condition is comparatively rare in my material, only eight cases having been observed. Fagerlind (1938a) did not see this stage. The nuclear reticulum does not completely reorganize, and no conspicuous vacuole is present between the two nuclei.

The second meiotic division follows quickly upon the first. The cell appears to be very little larger than at the time of the first division. The spindles of the second division commonly lie somewhat diagonal or at times nearly at right angles to the cell axis. Figure 10 shows in higher magnification the equatorial-plate stage seen in figure 9; figure 11 shows a late telophase stage in this division. The chromosomes are small and crowded.

Upon the completion of the second division, the resulting nuclei (fig. 12) often occupy positions approximately corresponding to the four points of a diamond. It is not uncommon to find them in a nearly linear arrangement (fig. 13). Rarely, all the nuclei lie closer to the chalazal end than is shown in figures 12 and 13. Such stages mark the beginning of the primary four-nucleate stage.

Soon after its organization, the micropylar nucleus is surrounded by a zone of dense cytoplasm (fig. 12, 13). This condition persists during the period of enlargement, which continues until this nucleus enters the prophases of the third division.

The three nuclei which lie closer to the chalazal end migrate toward that end (fig. 14). This movement is associated with a rapid elongation of the cell, and when the migration is nearly complete, conspicuous vacuoles appear (fig. 15). A large vacuole separates the three migrated nuclei from the micropylar one. A smaller vacuole usually appears between the micropylar nucleus and the micropylar end of the embryo sac. The three migrating nuclei continue their movement until they lie close together (fig. 16) at the chalazal end of the cell.

Fagerlind (1938a) points out that during the meiotic divisions the nuclei and spindles are dis-

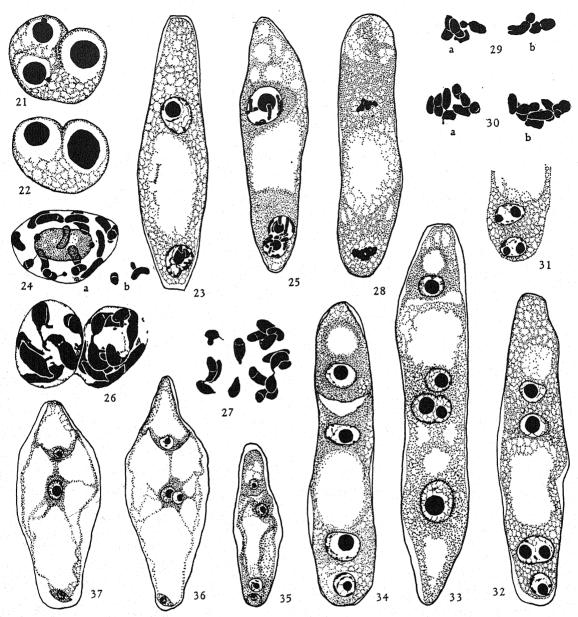


Fig. 21-37.—Fig. 21. Union of three chalazal nuclei.—Fig. 22. Union of three chalazal nuclei nearly complete.—Fig. 23. Embryo sac with nuclei in an early prophase of the third division; note the single lobed chalazal nucleus.—Fig. 24a, b. Adjacent sections of a triploid chalazal nucleus in prophase.—Fig. 25. Prophase of the third division, showing a diploid and a haploid nucleus uniting at the chalazal end.—Fig. 26. Haploid and diploid chalazal nuclei in late prophases of the third division, after disappearance of nucleoli. (The unusually large size of chromosomes is probably due to fixation in a Carnoy solution.)—Fig. 27. Polar view of chalazal nuclei in a very late prophase of the third division (one chromosome in an adjoining section).—Fig. 28. Entire embryo sac with division figures just before the equatorial-plate stage of the third division.—Fig. 29a, b, Adjacent sections of the micropylar division shown in fig. 28; enlarged.—Fig. 30a, b. Adjacent sections of the chalazal division figure shown in fig. 28; enlarged.—Fig. 31. Chalazal products of the third division uniting.—Fig. 32. Young secondary four-nucleate embryo sac; two nucleoli in one chalazal nucleus.—Fig. 33. A slightly older embryo sac with chalazal nuclei displaced toward the micropylar end.—Fig. 34. The usual secondary four-nucleate condition at the time of formation of the egg and antipodal cell.—Fig. 35. Enlarged embryo sac; fusion of the polar nuclei.—Fig. 36. Embryo sac at the time of the fusion of polar nuclei.—Fig. 37. Embryo sac; fusion of the polar nuclei completed.—Fig. 35-37 are × ca. 275; fig. 23, 25, 28, and 31-34 are × ca. 290; all others are × ca. 2330.

placed in the direction of the chalazal end of the embryo sac. Apparently he considers that this displacement, together with the later separation of the nucleus nearest the micropyle from the other three by a large vacuole, accounts for the location of three nuclei near the chalazal end of the sac. In general, there is some displacement of the nuclei and spindles as Fagerlind suggests; but as a rule it is not excessive and does not always occur (fig. 8, 12). My observations indicate that three of the four macrospore nuclei move almost to the chalazal end before the formation of a large central vacuole. The embryo sac is elongating at the time of formation of this large central vacuole, and both this elongation and the development of the vacuole probably contribute to the further separation of the chalazal nuclei from the micropylar nucleus.

The three chalazal nuclei now unite in various ways. In some cases two nuclei unite while a third lies very near (fig. 17-19). At other times, all three nuclei unite. In figure 21 all three nucleoli are still clearly visible, but the nuclear membranes in the planes of contact are disorganizing. It is probable that two of these nuclei have previously united. Figure 22 represents a more advanced stage of union. Meanwhile, as may be seen from figure 15, the micropylar nucleus has completed its growth and has entered the prophases of the third division. There is nothing to indicate that any of the uniting chalazal nuclei in these cases have advanced beyond the resting stage at this time.

Although chalazal nuclear unions may occur when the nuclei are in the resting condition, they take place as frequently in the prophases (fig. 20, 25, 26). A few unions, as will be seen later, are delayed until the metaphases, and even further delay is found in rare cases.

Very soon all nuclei have entered the prophases in preparation for the third nuclear division. The chalazal nuclei may now appear as three nuclei (comparatively rarely), as two (fig. 20), or most commonly as a single triploid nucleus (fig. 23, 24). In figure 23, the chalazal nucleus appears decidedly lobed as does also the single large nucleolus, but it is clear that there is only one nucleus. The nucleus shown in figure 24 gave a clear count of 18 chromosomes, two of which are in an adjacent section. Three satellite chromosomes are recognizable. In somatic equatorial plates (fig. 50), two satellite chromosomes are present. Thus it is clear that in the case illustrated by figure 24 the three chalazal nuclei have united into a single nucleus with 3 n (18)chromosomes. Figure 25 shows an embryo sac in which two chalazal nuclei (respectively 2 n and n) appear to be uniting in the prophases. The associated micropylar nucleus also is well advanced in the prophases.

The term "union" has been used in preference to "fusion" in the paragraphs preceding. When 18 chromosomes (3 n) are clearly present in one nucleus, there is no indication that the chromosomes of the component nuclei have become intermingled. Each component nucleus may still form a spindle of

its own (though it seems best to assume that only one spindle would be formed by this triploid nucleus). When a diploid nucleus is observed in the prophases, the chromosomes appear to be more or less distinctly separable into two groups. The nucleoli certainly unite in some cases. However, "fusion" would seem to imply a more intimate association of the nuclei than occurs here.

Nuclei in a more advanced stage of the third nuclear division are rarely encountered. The two chalazal nuclei (2 n and n) shown in figure 26 are in a late prophase after disappearance of the nucleoli. The unusually large size of these chromosomes is probably due to fixation in a Carnov solution. Figure 27 shows the chalazal complex in a very late prophase after the disappearance of the nuclear membranes. In this case it was possible to count 16 or 17 chromosomes on the section figured and one chromosome on an adjacent section. The chromosomes from one nucleus seem to be rather definitely grouped, whereas those from the other two nuclei are more closely associated. Apparently either two or three spindles would be involved in the ensuing division. The embryo sac shown in figure 28 has been reconstructed from three sections, and the chromosome groups found on the three sections are shown in figures 29 (the micropylar group) and 30 (the chalazal group). Figures 29 a and 30 b are from the same section. An examination of the groups shown in figures 30 a and b leads to the conclusion that 18 (3 n) chromosomes are in approximately the equatorial-plate stage, although no spindle fibers can be seen. (Compare figures 11 and 12, Fagerlind, 1938a.) It seems likely that two separate groups of chromosomes are participating in this chalazal division. Considering also stages like that shown in figure 24, where all the nuclei have united to form a single triploid nucleus, it seems very probable that one, two, or rarely three chalazal spindles may function in the third nuclear division.

Upon completion of this division, two triploid nuclei (fig. 34) are present at the chalazal end of the sac. In the majority of cases, each daughter nucleus contains only one nucleolus, and there is no indication that more than one spindle was involved. When two or three spindles are formed, they must commonly be so closely associated that the products unite in a single daughter nucleus at each pole of the spindle complex (fig. 31, 32). Each of the chalazal nuclei shown in figure 31 (after the third division) has two nucleoli, and the nucleus farther from the chalazal end is lobed. In several instances two nucleoli were observed in one of the chalazal nuclei (fig. 32). In one embryo sac, shown in figure 33, the division of the chalazal complex had probably involved two spindles and fusion of the daughter groups as previously described, but the chalazal division figure was located much farther than usual from the chalazal end of the sac. Chalazal nuclei with two nucleoli are never found at later stages, whence it appears that nucleolar fusion occurs in originally two-nucleolate nuclei.

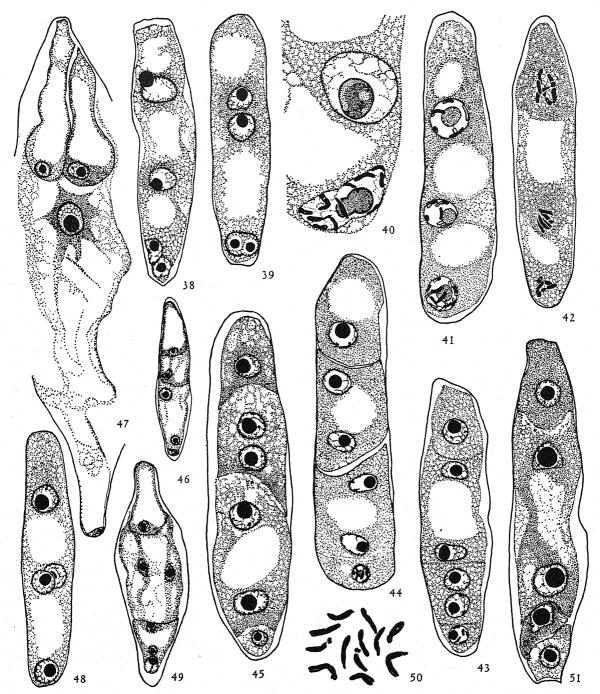


Fig. 38-51.—Fig. 38. Primary four-nucleate embryo sac at the time of migration of nuclei to the chalazal end; one nucleus lagging.—Fig. 39. Unusual case in which two chalazal nuclei have united, but the third lies near the micropylar nucleus.—Fig. 40. Union of two chalazal nuclei showing nucleoli uniting; a third chalazal nucleus is in a less advanced condition.—Fig. 41. Three widely separated nuclei entering the prophases of the third nuclear division; the chalazal nucleus is diploid.—Fig. 42. Three separate groups of chromosomes in late prophases (only part of the chalazal group is shown).—Fig. 43. Young six-nucleate embryo sac.—Fig. 44. Young four-celled six-nucleate embryo sac.—Fig. 45. Slightly older six-nucleate embryo sac in which the cell next the antipodal cell has begun to enlarge.—Fig. 46. Advanced five-nucleate embryo sac with a large cell next the antipodal cell.—Fig. 47. Mature embryo sac with a synergid-like cell.—Fig. 48. Primary four-nucleate embryo sac showing two nuclei lagging at the time of migration.—Fig. 49. Advanced six-nucleate embryo sac with the cell next the egg enlarging.—Fig. 50. Somatic chromosomes from root tip cell; 2 n=12.—Fig. 51. Peculiar five-nucleate embryo sac.—Fig. 46, 47, and 49 are × ca. 275; fig. 38, 39, 41-45, 48, and 51 are × ca. 920; fig. 40 and 50 are × ca. 2330.

After the completion of nuclear reorganization, the nucleus nearest the micropyle becomes the nucleus of the egg. The triploid nucleus next the chalazal end becomes the nucleus of the single antipodal cell. The remaining triploid chalazal nucleus and the remaining haploid micropylar nucleus are the

polar nuclei (fig. 33, 34).

There follows a period of enlargement during which the embryo sac grows considerably (fig. 35; this, as well as fig. 36 and 37 are drawn on a much smaller scale than the preceding figures). The two polar nuclei, respectively haploid and triploid, approximate and meet, commonly in the neighborhood of the egg. At the time of union of the polar nuclei (fig. 36), one nucleus (presumably the triploid one) is usually larger than the other, and commonly more chromatic granules are observable within it. A tetraploid primary endosperm nucleus results from this fusion (fig. 37). This primary endosperm nucleus remains in its position during the enlargement of the embryo sac which precedes fertilization.

Exceptional method of development.—A six-nucleate embryo sac was observed in one of my earlier preparations. This was of particular interest at the time, because it appeared to be a clear case of an exception to Dahlgren's story. Twelve other six-nucleate embryo sacs have since been found, as well as five five-nucleate sacs. Cases which appear to be exceptions to the regular course of events have been found also at earlier stages. A careful study of these preparations indicates that there is an alternative method of embryo-sac development in Plumbagella

micrantha.

An attempt was made to estimate the frequency of the occurrence of this type of development. Such an attempt is somewhat complicated by the occurrence of degenerating embryo sacs. It is possible that some embryo sacs which would develop into sixnucleate sacs degenerate instead. If this possibility be excluded, a count of all embryo sacs which have reached a comparable stage indicates that this alternative method is followed in about 5 per cent (16 in

300) of the ovules.

The exact origin of the six-nucleate embryo sacs is not entirely clear. It is possible that they may result from the postponement of meiosis in certain mother cells which (before meiosis) enlarge beyond the size ordinarily observed at this stage. Figure 2 is a moderate example of such enlargement. In another instance, the mother cell had grown to be about twice the ordinary size but still contained only one nucleus. If meiosis were to occur in an unusually large cell, it is conceivable that the third nuclear division might be initiated before three nuclei had migrated to the chalazal end. A six-nucleate sac could then result as explained below. On the other hand, it is possible that such enlarged mother cells merely are destined to degenerate.

The development of a six-nucleate embryo sac may be traced with reasonable certainty back to the time of migration of nuclei toward the chalazal end. At this stage certain cases (fig. 38) are observed in which two nuclei have moved to the chalazal end and

have become separated from the third nucleus by a vacuole, this latter nucleus being itself separated by a vacuole from the micropylar nucleus. The extreme condition (fig. 39) is rare. It is, of course, probable that the third nucleus at times succeeds in completing its migration before the third nuclear division takes place. However, it is certain that in some cases this nucleus fails to reach the chalazal region before it divides.

Very rarely two of the migrating nuclei are left behind the first and become separated from it by a vacuole (fig. 48). Under such conditions it is conceivable that the two lagging nuclei might enter the third nuclear division either as one (after union) or as two nuclei. There would then be present, before the third nuclear division, one haploid micropylar nucleus; a diploid central nucleus (or two haploid nuclei) and a chalazal haploid nucleus. No clear cases of a situation such as this, however, have been observed.

In case the third nucleus lags behind the other two, development proceeds as follows. The two nuclei which have migrated to the chalazal end come to lie very close together, or unite. Figure 40 shows these two chalazal nuclei in prophase with their nucleoli in process of uniting; the third chalazal nucleus is still at some distance and in a much less advanced condition. In such a case three rather widely separated nuclei (fig. 41) pass into division (or 4 if the two approximated nuclei at the chalazal end have not united). The micropylar nucleus has n (6) chromosomes, the central nucleus n, and the chalazal nucleus (or nuclei) 2 n. Figure 42 shows the three nuclei in a late prophase after the disappearance of their membranes. Upon completion of this division, the resulting embryo sac has two nuclei at the chalazal end each with 2 n chromosomes: two centrally located nuclei, each with n; and two micropylar nuclei each with n chromosomes (fig.

Cell division now occurs (fig. 43, 44). The micropylar nucleus becomes the nucleus of the egg; the diploid chalazal nucleus becomes the nucleus of the antipodal cell; the two haploid nuclei nearest the egg lie in a third cell and the two remaining nuclei in a fourth cell. This fourth cell, adjacent to the antipodal cell, has one diploid nucleus and one haploid nucleus. It would seem that in such an embryo sac there are two potential primary endosperm cells.

It is difficult in view of the limited material available to trace the further history of these six-nucleate sacs. As the embryo sac grows, it appears that in some cases the cell adjacent to the antipodal cell enlarges while the cell next the egg remains small (fig. 45). In the five-nucleate embryo sac represented in figure 46 (drawn on a smaller scale than the preceding figures), it appears that the cell next the egg has remained small but its two nuclei have united, whereas the cell adjacent to the antipodal cell has greatly enlarged and its nuclei are arranged much as are the polar nuclei at a corresponding stage in an embryo sac of the more usual type.

A single case, probably representing the final stage in this series, is shown in figure 47. Here it appears that the egg has elongated and pushed inward beside the adjacent (originally two-nucleate) cell. The nuclei in the latter cell have united; the resultant uninucleate cell is to all appearances a synergid. The nuclei in the two-nucleate cell adjacent to the antipodal cell also have united to form an apparent primary endosperm nucleus which in this case would have 3 n chromosomes. The remnants of the antipodal cell are present at the chalazal end.

In one instance (fig. 49) the two-nucleate cell adjacent to the egg has enlarged, leaving the other two-nucleate cell next the antipodal small and undeveloped. In neither cell have the two nuclei as yet united. Figure 51 may represent a case in which the nuclei of the cell next the antipodal have united. Later stages have not been found, but it would seem that, when fully developed, there would be one diploid antipodal cell and adjacent to it a cell with 3 n chromosomes having the appearance of an antipodal cell

Although the method of origin of these six-nucleate embryo sacs seems reasonably clear, it is difficult to explain the cause of their appearance. In all but one case, stages involved in their formation were obtained from Copenhagen plants which appear to have a stronger tendency toward a development of this type. Furthermore, this type of development was found only in material collected during July and August, when greenhouse temperatures were high. It is not unlikely that high temperature may be one factor contributing to this peculiar development.

The five-nucleate embryo sac shown in figure 51 may represent a case in which two chalazal spindles were widely separated and so arranged that the chalazal chromosomes from one spindle united with the micropylar chromosomes from the other spindle. Three chalazal nuclei would result. As already mentioned, it may also be a stage in the later development of a six-nucleate embryo sac. The former interpretation seems preferable, since this sac is smaller than six-nucleate sacs seen at a corresponding stage.

Discussion.—The complete omission of the fourth nuclear division is the major difference between the method of embryo-sac development herein reported for Plumbagella and that found in Fritillaria (Bambacioni, 1928a), Lilium (Bambacioni, 1928b; Cooper, 1935) and Tulipa (Bambacioni and Giombini, 1930). In Plumbagella the migrated nuclei (usually three) after the second meiotic division lie very close to the chalazal end of the embryo sac. The migration of these nuclei is possibly more rapid in Plumbagella than in the liliaceous genera mentioned, since some or all of them unite before they enter the prophases of the third nuclear division, whereas in Fritillaria, Lilium, and Tulipa the chalazal prophase nuclei form separate spindles which then fuse.

Fagerlind (1938b) reports that at least one species of Statice has a tetrasporic tetrapolar 16-nucleate embryo sac of the Penaea type (see Schnarf,

1936). He is in agreement with Haupt (1934) in considering the tetrapolar 8-nucleate *Plumbago* type of embryo sac as having arisen from the *Penaea* type. Since both types occur in the Plumbaginaceae, and since they show remarkable similarity in organization, the conclusion is logical.

According to Fagerlind, Armeria bupleuroides (?) has the Fritillaria type of embryo-sac development. He suggests that the Plumbagella type (as now known) may have arisen from the Fritillaria type; he suggests also that either the Plumbagella type has arisen from the Plumbago type or the Plumbago type has been derived from the Plumbagella type. For reasons which will be discussed below, an origin of the Plumbagella type from the Fritillaria type seems the more likely.

The limited geographical distribution of Plumbagella, combined with the fact that there is only one isolated species, would suggest that this genus is an old one. Also to be considered is the annual habit of P. micrantha, whereas Plumbago and Ceratostigma species known to me are perennial. The growth habit of Plumbagella also is quite distinct from that of most members of related genera. In certain characters, as type of inflorescence and growth habit, Plumbagella resembles Ceratostigma; in others it resembles Plumbago. This combination of features may likewise be interpreted as indicating an earlier origin of Plumbagella.

Chromosome studies of Phillips (1938) and my own unpublished observations have shown that the chromosome number (n=6) and chromosome morphology of this species are different from those of species of Plumbago (n=7 or 14). Maury (1886) considered Statice to be the most ancient genus of the family. It is noteworthy, therefore, that the chromosomes of Plumbago species and that they resemble more closely the chromosomes of species of Statice.

In published accounts, the Plumbago type of embryo-sac formation is established for Plumbago capensis (Haupt, 1934; Fagerlind, 1938a), P. zeylanica and Ceratostigma plumbaginoides (Dahlgren, 1937; Fagerlind, 1938a). The writer has made observations (unpublished) on embryo-sac development in P. capensis, P. zeylanica, P. scandens, P. coccinea, Ceratostigma plumbaginoides, and C. Willmottianum. The embryo sacs of all these species develop as does the embryo sac of Plumbago capensis. In all, the embryo sac is short and wide, whereas that of Plumbagella is long and narrow. Thus far it appears that Plumbagella is unique in its method of embryo-sac development.

The early history of the exceptional six-nucleate embryo sacs of *Plumbagella* approaches that of the embryo sacs of *Plumbago* more closely than does the history of the typical 4-nucleate sacs. The later behavior of these 6-nucleate sacs might be expected to show some resemblances to the story in *Plumbago* if the two types of development are nearly related. But the later history is entirely different in the two cases

Plumbagella micrantha appears to be an old, isolated species with a considerable period of separate evolution. Embryo-sac development resembles the Fritillaria type also found in the family. The only available evidence fails to indicate any link between the Plumbagella and Plumbago types. It would seem best, therefore, to consider that the Plumbagella and the Plumbago types have arisen independently—the Plumbagella type from the Fritillaria type, the Plumbago type from the Penaea type.

Some tendencies toward reduction have been reported for the Fritillaria type. Maheshwari (1937) summarizes the situation in the following sentences: "Of the two chalazal nuclei of the secondary 4-nucleate stage, the innermost is usually in a degenerating condition right from the time of formation. In Fritillaria, it succeeds in going through a normal division, but in Lilium (Cooper, 1935) the division is more or less abortive. In Gagea this division commonly does not take place at all and the embryo sacs are 7-nucleate; finally there are some cases in G. ova where both the chalazal nuclei fail to divide and the mature embryo sac is therefore only 6-nucleate. All three conditions may also occur in the same plant, viz., Myricaria germanica (Frisendahl, 1912)." Maheshwari considers Clintonia borealis to have a reduced embryo sac of the Fritillaria type. Reduction in all these cases involves only chalazal nuclei, whereas in Plumbagella the number of both chalazal and micropylar nuclei is reduced.

Since Dahlgren (1916) has reported the Adoxa type for eleven different species among the Staticeae, it is only fair to accept his decision until more evidence is available.

It is suggested, therefore, that development according to the *Penaea* type is the primitive method of embryo-sac development in this family. The *Plumbago* type can be directly derived from this by the elimination of the last nuclear division. The *Adoxa* type could be derived from the *Penaea* type. The *Fritillaria* type may be considered a modifica-

tion of the *Penaea* type and the condition in *Plumbagella* a reduction of the *Fritillaria* type resulting from the elimination of the last (fourth) nuclear division.

The evidence for such a view is by no means conclusive. However, it is hoped that these suggestions may be helpful in considerations of the phylogenetic relationships of the various methods of embryo-sac development found in the Plumbaginaceae.

#### SUMMARY

The egg nucleus in *Plumbagella micrantha* is separated from the nucleus of the macrospore mother cell by three divisions.

In general, three of the macrospore nuclei (resulting from the second meiotic division) migrate to the chalazal end of the embryo sac where two or three may unite. If union is not completed before division, it is finally affected on the spindle or in the formation of the two daughter nuclei. One macrospore nucleus remains at the micropylar end and divides at the same time as the newly formed chalazal complex.

Of the four nuclei thus formed, one (with n chromosomes) becomes the nucleus of the egg; another (with 3 n chromosomes) becomes the nucleus of the single antipodal cell; and the remaining two nuclei (with n and 3 n chromosomes, respectively) unite to form a tetraploid primary endosperm nucleus.

The development of anomalous six-nucleate embryo sacs results if one of the three nuclei ordinarily migrating to the chalazal end remains behind and divides independently.

The Plumbagella and Plumbago types of embryosac formation are considered to have arisen sepa-

The *Plumbagella* type is considered to have arisen as a modification of the *Fritillaria* type.

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# THE EFFECT OF VARIOUS COMPOUNDS UPON STRAIGHT GROWTH OF THE AVENA COLEOPTILE <sup>1</sup>

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IN THE course of the past two years we have tested one hundred and eighty compounds for their growth stimulating ability, using the method described by Scheer (1937). They were selected from a wide variety of structural types, and many were previously known to be physiologically active.

In the past three years more than fifty growth stimulating compounds, other than those reported here for the first time, have been recorded by Kögl and associates (1935, Mitt. 14, 16), Zimmerman and Wilcoxon (1935), Zimmerman and Hitchcock (1937), Koepfli, Thimann, and Went (1938), etc. Different test methods were used by each group of workers. The compounds reported on were effective in concentrations ranging from a few gamma to more than one hundred milligrams per liter. Certain of them are known to occur in nature, though most are synthetic and have not been detected in living tissue. However, they all affect the sum total of the processes known as growth.

For each compound we have studied, a series of concentrations has been tested with a view to determining the stimulating range of such substances as were found physiologically active. The same kind of test organism has been used throughout, though we recognize that this test plant may not be equally sensitive to the many different classes of substances worked with.

Although many of the substances we are reporting on are growth stimulators or growth inhibitors, no mention should be made of them as "phytohormones," at least at this time. Some are synthetic compounds, and others are naturally occurring substances belonging definitely to chemical categories other than that in which the now-known hormones appear.

Materials and methods.—The sources of the compounds studied are given in tables 1, 2, and 3. Any significant estimation of impurities which might be present and thus affect the physiological activity of the compound is difficult. Although the compounds tested were as pure as could be readily obtained, there may have been impurities capable of playing a part in the growth response.

Substances of widely different molecular structure characterize the compounds chosen. These include aliphatic, aromatic and heterocyclic compounds, and some of their derivatives.

A wide range of dilutions was tested in most instances. The concentrations were chosen after the response in the preliminary tests had been ascertained.

In nearly all instances, the compounds were dissolved in distilled water, and all dilutions were made from the original solution with a minimum of

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delay. Substances very slightly soluble in water were first dissolved in a few drops of absolute ethyl alcohol and then added to water. Solutions of given dilutions were then mixed with equal parts of 3 per cent agar. (All agar was washed in daily changes of distilled water for a period of two weeks before use.) Following this, the agar dilutions were poured

Table 1. Compounds promoting growth at dilutions tested.

(See table 4 for responses of test plants to different concentrations of compounds.)

	///	Range tested in	
Compound	Type ompound	milligrams per liter	Source
Abietic acid	Ring	0.08-100	3
p-Acetylaminobenzoic acid	Ring	0.08-100	3
Adenine	Ring	0.08-200	7
Aesculin	Ring	0.02-50	3
Aminosalicylic acid	Ring	0.08-100	3
α-Anilinopropionic acid	Ring	0.02-100	3
α-Anilino-n-valeric acid	Ring	0.08-100	3
Anisic acid	Ring	0.08-100	3
l-Asparagine	Chain	0.08-100	7
Cysteine hydrochloride	Chain	0.08-100	3
Glutathione	Chain	0.08-100	7
Glycolic acid	Chain	0.01-100	3
n-Heptylic acid	Chain	0.01-100	3
Hexylbutyric acid	Chain	0.08-100	3
Histamine biphosphate	Ring	0.08-200	7
Homophthalimide	Ring	0.01-100	3
Hypoxanthine	Ring	0.08-100	7
Indole butyric acid	Ring	0.08-200	2
Indoleacetic acid	Ring	0.02-200	2
Indolepropionic acid	Ring	0.08-100	2
Kynurenic acid	Ring	0.08-100	$\tilde{7}$
Lactic acid	Chain	0.08-100	3
Lauric acid	Chain	0.08-100	3
d-Leucylglycine	Chain	0.08-100	7
Maleic acid	Chain	0.08-100	3
	Chain		
Mucic acid		0.08-100	3
	Ring	0.02-200	2
a-(a-Naphthylamine) pro-		0.01.10	_
pionic acid	Ring	0.01-10	3
d-Phenylalanine	Ring	0.08-100	3
l-Phenylalanine	Ring	0.08-100	3
Potassium indole acetate	Ring	0.02-200	2
Potassium indole proprio-		43 41 <u>8 1</u> 22	3.78 (2.78)
nate	Ring	5–200	2
Potassium indole butyrate	Ring	0.02-200	2
Potassium naphthylacetate	Ring	0.02-200	2
d-Valine	Chain	0.08-100	7
<i>l</i> -Valine	Chain	0.08-100	7
Xanthine	Ring	0.08 - 100	7

<sup>&</sup>lt;sup>a</sup> Legend for tables 1, 2, and 3: 1, Abbott Laboratories; 2, Boyce Thompson Institute for Plant Research; 3, Dow Chemical Co.; 4, Eimer and Amend; 5, Merck Chemical Co.; 6, Pfanstiehl Chemical Co.; 7, Hoffmann-LaRoche; 8, Eastman Kodak Co.; 9, Mallinckrodt.

Table 2. Compounds inhibiting growth at dilutions tested.

Compound	Type compound	Range tested in milligrams per liter	Greatest inhibition	Source
Acetylcholine chloride	Chain	0.08–100	0.08-100	7
		0.08-100	50-100	3
Acetylcresotinic acid	Ring			3
Adipic acid	Chain	0.08-100	25-100	
Acetyltannic acid	Ring	0.08-100		3
l, l-Alanine	Chain	0.02-50	0.3	3
Aminocaprylic acid	Chain	0.08-100	25-100	3
-Aspartic acid	Chain	0.08-100	1.25-100	8
Barbital-sodium	Ring	0.04-200	12–200	5
Barium chloride	Inorganic	0.08-100	25	4
Benzoylbenzoic acid	Ring	0.08-100	0.08-100	3
Cadaverine dihydrochloride	Chain	0.08-100	0.08-100	7
Caproic acid	Chain	0.08-100	25-100	3
2-Carboxyphenoxyacetic acid	Ring	0.02-100	25-100	3
Carminic acid	Ring	0.02-200	10-200	3
Cinchonine hydrochloride	Ring	0.08-100	0.08-0.3	5
Colchicine	Ring	0.01-10	0.3-10	4
Cuprous chloride	Inorganic	0.002-100	50-100	4
Diamylamine	Chain	0.08-100	25	3
Digitalin	Ring	0.08-100	25-100	7
Digitanin	Ring	0.08-100	10-100	7
	Ring	0.08-100	5-100	3
Dihydroxybenzylacetic acid			50-100	7
Dimethylguanidine chlorhydrate	Chain	0.08-100		
Dimethylhexalinoic acid	Ring	0.08-100	25-100	3
Dinitro-o-cyclo-hexylphenol	Ring	0.004-100	0.3-100	3
Dithiosalicylic acid	Ring	0.08-100	10–100	3
Eosine yellow	Ring	0.08-50	0.3-50	• •
Fluorescein	Ring	0.08-25	10–25	3
Furacrylic acid	Ring	0.08-100	10–100	3
d-Glutamic acid	Chain	0.08-100	50-100	7
α-Glycerylsalicylate	Ring	0.08-100	10	3
Glycocyamine	Chain	0.08-100	10	7
Glycylglycine	Chain	0.08-100	50	7
Homophthalic acid	Ring	0.08-100	5	3
Indole	Ring	0.02-50	8-50	3
Mercuric chloride	Inorganic	0.004-5	0.01-5	4
d, l-Methionine	Chain	0.08-100	0.08-100	3
	Ring	0.04-100	0.04-100	6
Nucleinic acid	Ring	0.02-10	1.25	4
Papaverine hydrochloride	Enzyme	0.08-10	2-2.5	6
Pepsin		0.02-100	25-100	3
Phenoxyacetic acid	Ring		25-100 25-100	3
a-Phenoxypropionic acid	Ring	0.08-100		2
Phenylacetic acid	Ring	0.02-100	1.25-100	
Picramic acid	Ring	0.08-100	5-100	3
Pierie acid	Ring	0.08-100	50-100	3
Skatole	Ring	0.08-100	0.08-100	3
Sodium chloride	Inorganic		0.004-5	4
Taurine	Chain	0.08-100	25-100	7
Thiourea	Chain	0.04-100	0.08-25	6
Uric acid	Ring	0.04-100	0.08-100	7
Urea		0.04-200	5-200	6

a See footnote, table 1, for legend.

into molds of  $10.7\times8\times1.5$  mm., and each of these was cut into six small blocks of equal size, ready for application to the test plant. The Avena test plants were cultured and tested in a laboratory maintained at 25°C., 85–90 per cent relative humidity, and illuminated only with phototropically inactive light.

The test procedure used was that described by Scheer (1937) and consisted, in brief, of decapitating the coleoptiles of four-day-old Avena seedlings (ca. 20-25 mm. in length) to 16 mm., measured from the coleoptilar node (which was marked by a fine line of lamp black in paraffin oil). The enclosed foliar leaf was withdrawn. At the end of forty minutes, the agar blocks were applied across the terminal ends of the coleoptile stumps (fig. 1). A minimum length of time was allowed to elapse between

Table 3. Compounds ineffective at dilutions tested (or giving inconsistent results\*).

Range tested in Type milligrams per liter Sourcea Compound compound \*Agmatine sulfate ...... Chain 0.08 - 1007 0.08-100 7 \*Allantoin ..... Chain 7 Alloxan ..... Ring 0.08 - 100Chain 0.08 - 1003 \*l-Amino-iso-butyric acid. 0.08 - 1003 m-Aminobenzoic acid .... Ring Apomorphine hydrochlo-9 ride ..... Ring 0.01 - 107 0.08 - 100Arcaine sulfate ..... Ring 7 0.02-100 d-Arginine ..... Chain 0.08 - 1001 Ascorbic acid ..... Ring 3 0.02 - 50Barbituric acid ..... Ring Ring 0.08 - 1008 \*Betaine hydrochloride ... Inorganic 0.08-100 Cadmium chloride ...... 4 Calcium chloride ...... Inorganic 0.08-100 Inorganic 0.08-100 Chromic chloride ..... 4 Cobalt chloride ..... Inorganic 0.08-100 Caffeine hydrochloride.... Ring 0.08 - 1004 0.08 - 100Chlorophyll (H<sub>2</sub>O soluble) \*Cholic acid ..... Ring 0.08 - 1009 Cocaine hydrochloride .... 0.08 - 100Ring \*Cocaine salicylate ...... Ring 0.01-10 5 Codeine hydrochloride .... Ring 0.01 - 109 0.08-100 9 \*Codeine phosphate ..... Ring 9 0.08-100 Codeine sulfate ..... Ring 0.08 - 2007 Creatinine hydrochloride... Ring 0.02 - 1007 Creatine ..... Chain Cupric chloride ..... Inorganic 0.08-100 4 \*d, l-Cystine ...... Chain 0.02 - 1007 Diastase ..... 6 0.08 - 100Dionin ..... Ring 0.01-105 l-Dioxyphenylalanine ..... Ring 0.08 - 1007 Fumaric acid ..... Chain 0.08 - 1003 Furfural ..... Ring 0.08 - 1006 3 Glycine ..... 0.08 - 100Chain Glycine anhydride ...... 3 Ring 0.08 - 100Guanidine carbonate ..... Chain 0.08 - 1003 0.08-100 7 Guanine ..... Ring l-Histidine ..... Ring 0.08 - 1007 Hordenine sulfate ...... 0.08 - 1007 Ring 8 o-Hydroxybenzyl alcohol.. Ring 0.08 - 100o-Hydroxyquinoline ..... 0.08-100 Ring 3 l-Hydroxyproline ...... Ring 0.08 - 1007 7 d-Isoleucine ..... Chain 0.08 - 100*l*-Isoleucine ...... 7 Chain 0.08 - 1007 d-Leucine ..... Chain 0.08 - 100l-Leucine ...... Chain 0.08 - 1007 \*l-Leucylglycine ...... 7 Chain 0.08 - 100Levulinic acid ..... 3 Chain 0.08 - 100Lithium chloride ..... Inorganic 0.08-100 4 \*d-Lysine dihydrochloride. 0.08 - 1007 Chain Manganous chloride ..... Inorganic 0.08-100 4 \*Mescaline sulfate ...... Ring 0.08-100 7 o-Methoxybenzoic acid .... Ring 0.08 - 1003 \*6-Methyl-2-phenylquinoline-4 carboxylic acid ... Ring 0.08-100 3 Morphine acetate ...... Ring 0.01 - 109 \*Morphine hydrobromate... Ring 9 0.01 - 10\*Morphine hydrochloride.. 9 Ring 0.01-10\*Morphine sulfate ...... Ring 9 0.01 - 10

TABLE 3. Concluded.

Compound co	Type n	Range tested in nilligrams per liter	Source
Mukobromic acid Naphthylic acid	Chain Ring	0.08–100 0.08–100	3
Nembutal		0.08-100	3
Neonal acid		0.08-100	1
Neurine bromide	Chain	0.08-100	7
Nicotinic acid	Ring	0.03-100	3
*d-Ornithine dihydrochlo-	rung	0.02-00	J
ride	Chain	0.02-100	7
Phenobarbital	Ring	0.02-100	5
Phenylthioglycine-o-carbox-			
ylic acid	Ring	0.08-100	3
Phthalonic acid	Ring	0.02-100	3
l-Proline	Ring	0.08-200	7
Putrescine dihydrochloride	Chain	0.08-100	7
Pyrogallic acid	Ring	0.08-100	3
Pyromucic acid	Ring	0.08-100	3
*Pyruvic acid	Chain	0.08-100	3
*Rubidium chloride	Inorganic	0.08-100	4
Quercitrin	Ring	0.02 - 50	8
Salicylsalicylic acid	Ring	0.08-100	3
Saponin	Ring	0.08-100	3
Sarcosine	Chain	0.08-100	7
Sarcosine anhydride	Chain	0.08-100	7
d, l-Serine	Chain	0.08-200	7
Spermine hydrochloride	Chain	0.08-100	7
Steapsin		0.08-100	6
Strontium bromide	Inorganic	0.08-100	4
Strontium chloride	Inorganic		4
Strontium nitrate	Inorganic		4
Strontium sulfate	Inorganic		4
Strychnine hydrobromide	Ring	0.08-100	4
Strychnine hydrochloride	Ring	0.08-100	4
Strychnine sulfate	Ring	0.08-100	4
*Tannic acid	Ring	0.02-100	3
Trypsin		0.08-100	6
Uranium acetate	Chain	0.08-100	4
*Vanillic acid	Ring	0.02-50	3
Zinc chloride	Inorganic		4

<sup>&</sup>lt;sup>a</sup> See footnote, table 1, for legend.

the preparation of the agar blocks and their application to the test plants. In every set of tests plain 1.5 per cent agar blocks were applied to 12 test plants (controls) as the basis for estimating the growth stimulating qualities of the compound tested. All measurements were made with a small millimeter rule eight hours after the application of the agar blocks, and the length was recorded to the closest quarter millimeter.

The growth in length of the coleoptiles to which agar containing known amounts of a substance has been applied, over the controls (plain 1.5 per cent agar), is expressed in table 4 as millimeters increase over agar control. One or more check runs were made on most of the active substances. Although the magnitude of the response varied from test to test, the results were of the same order; variations at different test periods were comparable to those

reported by numerous others in the Avena curvature response.

About twenty separate tests of given dilutions, selected at random, were examined for probable error. The maximum found was  $\pm 0.1$  mm.

In addition to tests on straight growth, all compounds were tested for their ability to promote curvature in *Avena* in the usual Went test (coleoptile curvatures within 110 minutes after application of blocks). Results showed that ten were capable of bringing about *Avena* curvatures (see table 4), and the most active of these were re-tested by the "deseeded" method of Skoog (1937) (see table 5).

Compounds promoting straight growth of the avena coleoptile.—The derivatives of 3-indoleacetic acid and a-naphthyleneacetic acid show greater activity than do the other compounds tested, as shown in the histogram (fig. 2). The potassium salts of the various acids were prepared from the same samples of acids here tested; the close agreement (over the proportionality range) in the results of tests on acids and salts see tables 4 and 5) sug-

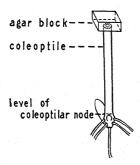


Fig. 1. Diagram of Avena seedling as test object in straight growth method (adapted from Scheer). The coleoptile is marked at the coleoptilar node, then decapitated to 16 mm. (the inclosed foliage leaf is withdrawn entirely). The agar block containing the substance to be tested is pressed gently over the entire cut surface of the coleoptile stump. Length is measured 8 hours after application of agar blocks (see text).

gests that the explanation for previous tests in which the potassium salts brought about a greater response than the acids (Avery, Burkholder, and Creighton, 1937; Scheer, 1937) lies in the fact that the salts were not prepared from the same samples of acids which had been used in their tests.

Amino acids, etc.—Of the amino acids, etc., tested and found active, tyrosine, tryptophane, p-acetylaminobenzoic acid, l-asparagine, l-valine, and xanthine produced the greatest response. This response, however, was not equal to that produced by certain other compounds, notably indoleacetic acid, naphthaleneacetic acid, and their derivatives.

The amino acids just mentioned were not equally effective at the same concentrations, as shown in figure 2 and table 4. The active range of *l*-valine is in the weaker concentrations while *d*-valine, *l*-phe-

<sup>2</sup> Delayed response.

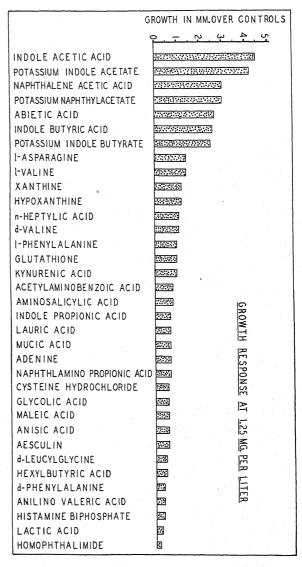


Fig. 2. Histogram showing straight growth response of the *Avena* coleoptile to the compounds listed in table 4, all compounds at a concentration of 1.25 milligrams per liter. The maximum response often came at higher concentrations, hence the histogram shows only comparative response at a given concentration.

nylalanine, and d-phenylalanine seem to promote over the whole range tested. The rest of the compounds promote mainly in the more concentrated range. The various types of responses, notably those of homophthalimide, l-valine and l-phenylalanine, indicate that, although all of them promote straight growth in Avena, they do so by very different means. It is possible that some of the compounds may be used by the coleoptile as accessory nutritive material.

Delayed response brought about by certain amino acids.—Agar made up with tryptophane and tyrosine and applied immediately to the test plants,

Table 4. Straight growth of the Avena coleoptile in response to different dilutions of various growth stimulating substances. All figures represent growth in mm. over agar controls. Measurements were made 8 hours after applying agar blocks.

	Number of Avena plants in the final		Dilutions promoting (mg. per liter) growth							Avena curva-		
Name of compound	test (per dilution)	0.02	0.08	0.3	1.25	5.0	10.0	25.0	50.0	100	200	ture afte two hour
AMINO ACIDS AND DERIVATIVES			٠.									
3-Indole acetic acid	12	1.4	3.9	4.2	4.5	5.0	5.2	4.4	4.1	5.1	3.5	+
Potassium 3-indole acetate	12	1.3	4.1	4.8	4.2	4.6	4.9	5.9	4.9	4.6	7.0	+
3-Indole butyric acid	12		0.7	2.2	2.6	2.7	3.5	2.6	3.0	3.1	3.7	+
Potassium 3-indole butyrate	12		0.4	1.4	2.5	3.2	4.2	4.9	4.5	3.4	2.7	+
a-Naphthaleneacetic acid (Merch			1.6	2.7	3.0	3.8	3.2	3.5	3.5	2.9	2.6	+
Potassium a-naphthylacetate			1.2	1.9	3.0	3.0	3.5	2.3	3.3	3.6	2.6	+ .
α-Naphthaleneacetic acid (Wil-												
coxon)			0.5	1.7	2.7	3.4	2.9	3.8	2.1	2.7	2.2	+
3-Indolepropionic acid		• •	0.8	0.5	0.7	1.3	1.0	2.0	2.3	2.4	• • •	+
Potassium 3-indolepropionate		• •	• •			-0.2	0.6	1.1	1.4	1.7	1.7	+
α-Anilinopropionic acid	24	• •				• • •	0.5	0.4	1.0	2.0		+
p-Acetylaminobenzoic acid	12	٠		0.0	0.8	0.9	1.5		0.9	0.4		0
d-Phenylalanine			0.3	0.2	0.4	1.5	1.5		1.2	0.9		slight
l-Phenylalanine			1.1	1.4	1.0	0.9	1.4	1.3	0.8	0.9		slight
l-Asparagine			1.1	0.9	1.4	1.0		0.7	1.6	0.7	٠	0
d-Valine			0.7	1.1	1.1	0.7	1.0	1.0		0.8		0
<i>l</i> -Valine			0.5	1.4	1.4	0.9	0.4		0.5	0.3		0
Xanthine			0.9	1.0	1.2	1.4		1.4	0.7	0.2		0
α-Anilino-n-valeric acid			0.7	0.7	0.4	0.4	0.4	1.2	0.6	0.4	• • •	0
d-Leucylglycine			1.0		0.5	0.7	0.6	0.4	0.7	0.4		0
Homophthalimide	12	• •		0.2	0.2	0.1	0.0	0.4	1.2	0.6	••	0
			0.3	0.3		0.1	1.0		0.3	0.3	• •	0
Aminosalicylic acid					0.8			1.1			• •	-
Glutathione			0.2	0.5	1.0		0.8	0.7	0.2	• • •	••	0
Cysteine hydrochloride	12		0.8	0.8	0.6	0.6	1.3	0.8	0.8	0.1	• • •	slight
FATTY ACIDS												
n-Heptylic acid	18	1.6	1.8	1.5	1.1		0.6	0.2	0.2	0.2		0
Hexylbutyric acid			1.5	1.4	0.5	0.5	0.6	0.2				0
Glycolic acid		0.8	0.9	0.6	0.6	0.7	0.3	0.5	0.3	0.3		0
Lauric acid			0.9	0.7	0.7	0.5	0.6	1.1	0.6	0.7		0
Mucic acid				0.6	0.7	0.4	1.4	0.7	0.4	0.4		0
Lactic acid			1.0	0.5	0.3	0.8	0.4	0.3	• • • • • • • • • • • • • • • • • • • •	0.2		0
Maleic acid			0.4	0.5	0.6	0.6	0.8	0.6			•	0
	12	• •	0.7	0.0	0.0	0.0	0.0	0.0	•	•••	•,•••	
MISCELLANEOUS												
Abietic acid		• • •	1.5	• • •	2.7	1.1	0.8	1.3	1.6	1.1	• •	0
Adenine		• •	0.2	0.4	0.7	0.4	0.5	0.5	0.6	0.7	1.1	0
α-(α-Naphthylamino) propionic a		0.8	1.0	0.8	0.7	0.2						0
Histamine biphosphate	18	• • • • •		0.3	0.4	0.6	1.0	0.3			• •	0
Kynurenic acid			0.8	0.5	1.0	0.6	0.6	0.5	0.5	0.4		slight
Hypoxanthine	18		0.9	1.0	1.2	1.4		1.4	0.7	0.2		0
Anisic acid	24		0.8	0.6	0.6	0.6	0.5	0.5	0.5	0.8		0
Aesculin	6		0.7	0.9	0.6		0.3					0

brings about no response. However, if the agar containing given amounts of these compounds is allowed to stand at laboratory temperatures (in the dark) for varying lengths of time before application, chemical changes occur, and an active compound or compounds are produced; the possibility of enzymes in the agar seems precluded by its preparation—i.e., the long period of washing, autoclaving, and keeping sterile until use. Data bearing on this will be published later. Skoog (1937) reported that tryptophane and indole ethyl amine, in agar, were "activated" (chemically altered) upon contact with

plant tissue for a given period of time. The fact that a number of compounds included in table 3 gave inconsistent results from test to test suggests the desirability of examining response in relation to time in a considerable number of compounds.

Fatty acids.—Several fatty acids and substituted fatty acids were tested, and although none of them were quite as active as the indole compounds, they nevertheless caused substantial promotion of growth (table 4). n-Heptylic acid was the most active of the group tested. It promoted growth markedly in the lower concentrations used. The range of concen-

Table 5. Avena tests by Skoog's (1937) deseeded method, photographs after five hours. Curvatures by this test method are (in this laboratory) ca. 4 × as great as in the Went method. Each figure is the average curvature for two dozen test plants.

			$\mathbf{Results} \; \mathbf{in}$	degrees Ar	ena curvatu	re	
Mg. per liter	Indole acetic acid	Potassium indole acetate	Indole butyric acid	Potas- sium indole butyrate	Naphtha- lene acetic acid (Merck)	Naphtha- lene acetic acid (Wilcoxon)	Potas- sium naphthyl acetate
0.01	5.1	5.1					
0.02	11.9	10.6					
0.04					5.6	5.3	4.5
0.08		• •	5.4	5.6	10.1	11.3	9.0
0.16			9.3	7.8			

trations in which they promote growth is, for the most part, lower than that in which the amino acids are particularly effective.

Miscellaneous. — Many compounds of varied structure were tested; they cannot be classified under any of the above headings. Only 8 were active. The most active of this group of miscellaneous compounds was abietic acid which gave good promotion at 1.25 mg. per liter (table 4). Certain rather complex molecules were effective, and the compounds seem to have no particular relationship, structurally, with the known growth promoting substances reported here or by previous workers.

Compounds inhibiting straight growth of the avena coleoptile.—Our main object at this time is to make available to others the results of our inquiry into compounds capable of growth stimulation, but growth inhibiting substances should by no means be overlooked. Indeed, studies of the influence of growth depressing effects of certain types of compounds may well provide the key to the processes being influenced by growth stimulating substances.

Compounds found to be inhibiting are given in table 2, together with the dilutions tested. It is possible that other than growth depressing effects might be found if these substances were tested at different concentrations.

Compounds neither promoting nor inhibiting straight growth of the avena coleoptile.—Ninety-three of the compounds were neither growth promoting nor growth inhibiting in the dilutions tested (table 3), although twenty of this number gave inconsistent results.

DISCUSSION.—It is clear from the data presented that a very diverse group of substances possess the capacity to stimulate growth. Many of them do not have the configuration suggested as characteristic of "phytohormones" by Koepfli, Thimann, and Went (1938), though several of the compounds reported here show greater effectiveness in growth stimulation, as compared with 3-indoleacetic acid, than

<sup>3</sup> This statement is based on the consideration that if 3-indoleacetic acid at its optimum concentration gives 100 per cent response in straight growth, then all the compounds we list as active bring about at least 11 per cent of this response at their optimum concentration. Koepfli, Thimann, and Went, utilizing the pea test, con-

some of the compounds listed as active in their study. This raises several questions which can be only partly answered:

(1) Are the numerous compounds shown to be physiologically active in such tests as the ones used in this study, or any other, necessarily "phytohormones"? Went and Thimann's modification of Bayless and Starling's definition of a hormone is satisfactory for the purpose of discussion: "a substance which, being produced in any one part of the organism, is transferred to another part and there influences a specific physiological process." On the basis of this definition, only naturally occurring compounds may be regarded as hormones. Hence, until such substances as are dealt with here are demonstrated to be present in plant tissue, we must think of them only as "growth substances." They are, of course, physiologically akin to the naturally occurring hormones reported present in higher plants. The point presented here in connection with the delayed action of tryptophane and tyrosine (both known to occur abundantly in higher plants) suggests that many different naturally occurring hormones may be produced from amino acids (Avery, et al., unpublished except for abs.). The fact that growth substances produced from the amino acids are short-lived will make it difficult to isolate and identify them. Owing to the diversity of the active compounds, we can conclude little beyond what is already known-protoplasm responds to many different kinds of substances. However, the quantitative study presented here should clarify somewhat the physiological relationships of the many substances which might mistakenly be regarded as 'phytohormones."

(2) Do the active compounds have any molecular configuration in common? The more active substances do, but many of the less active, or even inhibitory compounds, are also structurally similar to the highly active ones.

(3) Why do not the compounds promoting straight growth cause curvature when tested by the curved-growth technique of Went? The lack of positive results in the curvature tests may be due to these sidered as active those compounds giving down to 2 per cent of the indole acetic acid response.

compounds having to undergo chemical alteration and to the fact that the physiologically active intermediate or end products have not been formed within the approximately two-hour-time lapse of the curvature test. If our tests had been made with the curved-growth technique only, most of the compounds would have been judged inactive. The greater length of time used in the straight growth technique would allow such changes to take place and time for the new compounds to take effect before the results are recorded. Failure to get the curved growth response with most compounds might also be attributed to the possibility that most compounds unilaterally applied in agar quickly become evenly distributed through the coleoptile, instead of remaining unilaterally distributed.

(4) Do compounds synthesized at different times, or by different methods, differ appreciably in their physiological effectiveness? Previously obtained data (Avery, Burkholder, and Creighton, 1937; Zimmerman and Hitchcock, 1937) make it appear, in the light of the present tests, that such may be the case for indoleacetic and indolebutyric acids and their potassium salts. In the present tests, the potassium salts were prepared from the same sample of acid tested, and the results were comparable in both the straight and curved growth tests.

Straight and curved growth tests were also made on two samples of naphthaleneacetic acid, one synthesized by Merck and Co., the other by Wilcoxon (1937). Both were obtained through the courtesy of P. W. Zimmerman. Although the physical appearance of the two preparations is different, their melting points are the same. The results of the straight growth tests show somewhat lower activity in the Wilcoxon preparation, whereas in the curved growth tests they appear approximately the same. Dr. Zimmerman reports by correspondence that with the green tissue test objects the two preparations were equally effective.

(5) The nature of the test object. The various test methods have their merits according to the

problem being investigated, and while this study has been primarily one on compounds, the kind of test object is a matter of importance which must not be entirely overlooked. The straight and curved growth Avena tests used here involve the use of etiolated Avena seedlings. The pea test of Went involves the use of etiolated Pisum seedlings (Went and Thimann, 1937). In contrast to these are the green tissue test objects used and discussed by Hitchcock and Zimmerman (1938, etc.), and others. Such test objects have been used in studies on the induction of roots as well as in other physiological responses. In the case of root induction the compounds being tested initiate cell division. However, cell elongation is involved in bringing about nastic and tropic responses, whether in green tissue or etiolated test objects.

In the case of indoleacetic acid, straight growth of the Avena coleoptile is promoted over a range in concentration of 20,000 fold. Other substances are less active in this particular test, most of them promoting growth over a much less extensive range of concentration. Hitchcock and Zimmerman note a range in concentration of 10,000 fold in which numerous substances are effective in inducing root formation in green tissue test objects.

#### SUMMARY

One hundred and eighty compounds of widely different molecular structure were tested for their ability to stimulate straight and curved growth of the Avena coleoptile. Aliphatic, aromatic, and heterocyclic compounds and some of their derivatives were tested. At the dilutions tested, 37 stimulated growth, 50 inhibited growth, and 93 were without apparent effect or gave inconsistent results. Only 10 of the active compounds—these previously well known—gave a clear-cut Avena curvature response.

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other responses in plants. Contrib. Boyce Thompson Inst. 7: 209-229.

# TWO NEW SPECIES OF RANUNCULUS § FLAMMULA 1

Lyman Benson and Annetta Carter

SINCE Latin descriptions are not considered to be desirable in a manual, the impending publication of volume 2 of Dr. L. R. Abrams' Illustrated Flora of the Pacific States makes necessary advance publication of two new species of Ranunculus, subgenus Euranunculus, section Flammula, as follows:

Ranunculus oresterus Benson, sp. nov. Practically glabrous terrestrial perennials; roots very numerous, filiform, 0.5-1 mm. in diameter, each root densely covered with hairs for its entire length; stems erect, not rooting adventitiously, about 1 dm. long and 1-1.5 mm. in diameter, 2-4-flowered, somewhat fistulose, glabrous except for long appressed stout hairs at the bases; radical leaf-blades simple, each broadest at the middle and tapering to the two acute ends, 3-4.5 cm. long by about 6-7 mm. broad, entire, glabrous, the petioles 2-4 cm. long, glabrous, markedly dilated, 1-3 mm. broad, the dilated leafbase perhaps forming almost the entire petiole, cauline leaves alternate or almost opposite, 2-5.5 cm. long by 2-8 mm. broad, sessile, some bracts opposite, sessile; pedicels 1.5 cm. long in flower and 2-5 cm. long in fruit, glabrous; sepals vellowish and petaloid, spreading, narrowly oblong, 3 mm. long by 1-1.5 mm. broad, glabrous, the length of the petals; petals 5, yellow, narrowly oblong, 3 mm. long by 1-1.5 mm. broad, the nectary-scale glabrous, apparently forming a pocket; stamens perhaps 5-10 (5 visible in the single flower); achenes 15-25 in a depressed-globose head 2-4 mm. long by 2.5-4 mm. in diameter, each achene obovoid or oblong-obovoid, 1-1.5 mm. long by 0.7-1 mm. dorsoventrally by 0.7-0.8 mm. laterally, smooth, glabrous, the margin with a distinct nerve, the achene-beak filiform, 0.4-0.6 mm. long, not curved; receptacle subglobose, 1 mm. long in flower and 1.5-2.5 mm. long in fruit, glabrous.

Herba terrestris perennis glabra; radicibus filiformibus pubescentibus; caulibus 1 dm. longis, 1-1.5 mm. diametro; foliis petiolatis integerrimis lanceolatis vel oblanceolatis 3-4.5 cm. longis, 6-7 mm. latis; sepalis 3 mm. longis, 1-1.5 mm. latis; petalis 5 luteis oblongis 3 mm. longis, 1-1.5 mm. latis; staminibus 5-10; carpellorum capitulo globoso 2-4 mm. longo, 2.5-4 mm. diametro; acheniis 15-25 obovatis laevibus 1-1.5 mm. longis; rostro filiformi 0.4-0.6 mm. longo.

In swales at the summit of the Blue Mountains, eastern Oregon, altitude 4,000 feet (fide Washington State College specimen), Baker City-Canyon City road. Known only from the type collection by

<sup>1</sup> Received for publication May 22, 1939. <sup>2</sup> As delimited by Benson. 1936. Amer. Jour. Bot. 23: 26-33, 169-176. William C. Cusick, June 4, 1902, No. 2800. Type specimen in the Dudley Herbarium, Stanford University, California, No. 96453. Isotypes: University of Oregon Herbarium, 3 sheets marked by the writer in 1932 with a square containing an x in its lower left corner; Washington State College Herbarium No. 10201; New York Botanical Garden.

Ranunculus oresterus is most closely related to R. alismaefolius Geyer and its varieties, particularly the variety montanus S. Wats. (Ranunculus calthaeflorus Greene.) It is to be most readily distinguished by (1) its pubescent roots (densely hairy their whole length), by (2) its remarkably small flower,<sup>3</sup> the petals not exceeding the sepals and being only 3 mm. long and 1–1.5 mm. broad, by (3) its small size, the stems being only 1 dm. long and 1–1.5 mm. in diameter and 2–4-flowered (R. alismaefolius var. alismellus A. Gray is almost as small, but is distinguished readily by ovate instead of elongate leaves and by ovate instead of narrowly oblong petals about 6 mm. long), and (4) by having only about 5 or 10 (5 visible in the flower) stamens.

Ranunculus alveolatus Carter, sp. nov. Glabrous to sparsely pubescent, semi-aquatic annual; roots filiform, numerous; stems many-branched from near the bases, semi-decumbent to erect, rooting adventitiously at the lower nodes, 12-30 cm. long and 1-1.5 mm. in diameter, fistulous; leaves alternate, the blades simple, ovate to ovate-lanceolate, 6-20 mm. long by 4-12 mm. broad, entire to slightly dentate, petioles of lower cauline leaves 30-85 mm. long, petioles of upper cauline leaves 6-35 mm. long; stipular leaf-bases membranous-margined, non-ciliate or slightly ciliate at the apices, 4-6 mm. long, conspicuous; pedicels 5-10 mm. long in flower and 10-40 mm. long in fruit; sepals 3, membranous-margined at the bases, broadly to obtusely ovate, 2-2.5 mm. long by 1-1.5 mm. broad, glabrous, persistent; petals 2-3, yellow, clawed, each blade ovate, 2-2.5 mm. long by 1 mm. broad, the nectary-scale a glabrous pocket, truncate; stamens 4-5, 1.25-1.5 mm. long; achenes 15-25 in a slightly elongated head 3-5 mm. long by 3-4 mm. in diameter, each achene oval, flattened, 1.5 mm. long by 1.25 mm. dorsoventrally by 0.5 mm. laterally, the surface alveolate, the margin obscure, the achene-beak obsolete; receptacle conical to narrowly ovate, 1 mm. long in flower and 2-3.5 mm. long by 1.25-1.5 mm. in diameter in fruit, glabrous.

Herba annua amphibia, glabra vel sparse pubescens; radices filiformes; caules 12-30 cm. longi,

<sup>3</sup> Unfortunately only one flower is available (the isotypes have abundant fruit, but no flowers), and, consequently, publication has been delayed ten years in hopes of obtaining more material.

1-1.5 mm. diametro, fistulosi, semidecumbentes vel erecti, ad basim versus multiramosi, ramis e nodis inferioribus radicantibus; folia alterna laminis ovatis ovato-lanceolatisve integerrimis vel paullo dentatis; foliorum inferiorum petioli 30-85 mm. longis, superiorum 6-35 mm. longis; laminae 6-20 mm. longae, 4-12 mm. latae; stipulae 4.6 mm. longae, conspicue membranaceo-marginatae eciliatae vel sparse ciliatae; pedicelli per anthesin 5-10 mm. longi, fructiferi 10-40 mm. longi; sepala 3, late obtuseque ovata, basi membranaceo-marginata 2-2.5 mm. longa, 1-1.5 mm. lata; petala 2-3, lutea unguiculata 2-2.5 mm. longa, laminis ovatis 1 mm. latis, squamulis nectariferis truncatis; stamina 4-5; carpellorum capitulum ovoideum, 3-5 mm. longum, 3-4 mm. latum; achenia ovalia complanata 1.5 mm. longa, diametro dorsoventraliter 1.25 mm., lateraliter 0.5 mm.; pericarpio alveolato, rostro obsoleto; receptaculum in fructu 2-2.5 mm. longum, 1.25-1.5 mm. latum.

Margins of ponds and marshy areas along small streams; Sierra Nevada foothills from Calaveras County to Placer County, California. The type specimen was collected between Fair Oaks and Folsom, 11/2 miles southeast of Orangevale, Sacramento County, April 25, 1937, Annetta Carter 1244 (Herbarium of the University of California No. 604080).

The following material from Dudley Herbarium, Stanford University (SU) and the Herbarium of the University of California (UC) has been examined. Placer County: Roseville, J. W. Congdon, April 2, 1894 (SU). Sacramento County: Folsom, Katherine Brandegee, May 8, 1907 (UC, in part); between Fair Oaks and Folsom, 11/2 miles southeast of Orangevale, Annetta Carter 821, May 12, 1935 (UC). Calaveras County: 7.5 miles west of San Andreas, John Thomas Howell 4707, April 13, 1930 (SU, UC).

The relationships of the two new species to other members of the section Flammula occurring in North America north of Mexico are shown by the following kev:

Perennials; achenes 1.2-2.5 mm. long, the beaks 0.3-1.5 mm. long or in one species 0.1-0.2 mm. long; petals 5-10, conspicuous and usually large, longer than the sepals, except in one species.

Subsection 1. FLAMMULAE. Achenes nearly beakless, the beaks, if present, 0.1-0.2 mm. long, the styles stout. 1. R. Flammula. Achenes distinctly beaked, the beaks 0.3-1.5 mm. or rarely only 0.2 mm. long.

Cauline leaves lanceolate, oblanceolate, or linear.

Roots glabrous in their mature portions; petals exceeding the sepals.

Leaves not dentate, sometimes serrulate or wavvmargined, usually entire.

Stems rooting at at least the lower nodes.

Achene beaks 0.3-0.5, rarely 0.7 mm. long; stems mostly less than 2.5 mm. in diame-1. R. Flammula vars.

Achene beaks 1 mm. long; stems mostly 5-10 2. R. ambigens. mm. in diameter.

Stems never rooting adventitiously.

3. R. alismaefolius.

Leaves (at least the radical) dentate.

5. R. hydrocharoides var.4

Roots densely pubescent their whole length; petals 5, not exceeding the sepals, narrowly oblong, 3 mm. long by 1-1.5 mm. broad; leaves entire; stems not rooting adventitiously.

4. R. oresterus.

Cauline leaves ovate or ovate-lanceolate.

Stems rooting at at least the lower nodes; flowers never in cymes.

Roots with no thickened storage parts.

Stems not fistulous or inflated, 1-1.5 mm. thick; cauline petiols sheathing the stem.

1. R. Flammula var.

Stems usually fistulous, 1-5 mm. thick, cauline petioles not sheathing the stem.

5. R. hydrocharoides.

<sup>4</sup> A variety the recombination of which is now in press. Based upon R. stolonifer Hemsl.

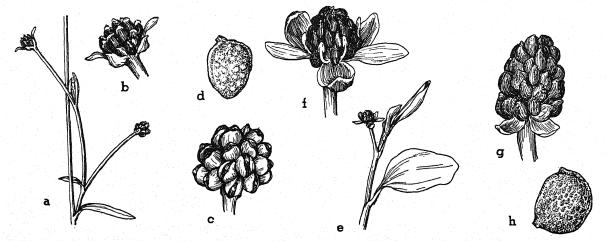


Fig. 1. Ranunculus.—Fig. a-d, R. pusillus: a, upper portion of plant showing sessile, linear-lanceolate leaves, X3; h, flower, ×9; c, hemispherical fruiting head, ×8; d, papillate achene, ×10.—Fig. e-f, R. alveolatus: e, upper portion of plant showing petiolate, ovate leaves,  $\times 2$ ; f, flower,  $\times 9$ ; g, fruiting head,  $\times 6$ ; h, alveolate achene,  $\times 10$ .

Roots each with a light-colored, fusiform storage thickening at the base. 6. R. Gormanii. Stems never rooting; flowers usually in cymes; roots each with a light-colored fusiform storage thickening at the base. 7. R. Populago. Annuals; achenes 0.6-1 or rarely 1.5 mm. long, the beaks 0.1-0.2 mm. long. Subsection 2. PUSILLI. Petals 5-9, large and conspicuous, about twice as long as the sepals; styles in anthesis 0.5 mm. long, filiform, deciduous in fruit; head of achenes hemispherical or ovoid. 8. R. oblongifolius. Petals 1-3, minute and inconspicuous, shorter than or equal to the sepals; styles in anthesis 0.1-0.2 mm.

Sepals 5; upper cauline leaves linear to lanceolate or oblanceolate or very narrowly elliptic, sessile;

achenes oblong-obovate, 0.6-1 mm. long, smooth, reticulate, or papillate, the units of the reticulate pattern perhaps 0.03 mm. in diameter when visible.

9. R. pusillus.

Sepals 3; upper cauline leaves ovate, petioled; achenes obovate, about 1.5 mm. long, reticulate, the units of the pattern about 0.1 mm. in diameter.

10. R. alveolatus.

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## THE INHIBITION OF ROOT GROWTH BY AUXINS 1

James Bonner and J. B. Koepfli

It was shown by Kögl, Haagen-Smit, and Erxleben (1934) that solutions of crystalline auxin-a, or of indoleacetic acid greatly retard the growth of roots. This effect appears to be due to a specific property of auxins rather than to mere toxicity, since concentrations of indoleacetic acid as low as 0.005 mg. per liter suffice to give a detectable inhibitory effect upon the growth of wheat roots (Marmer, 1937).

It would seem paradoxical that auxins, which exert a promotive effect on the growth of stems, petioles, coleoptiles, etc., should, in concentrations of the same order of magnitude, exert exactly the reverse effect on the growth of roots. Numerous studies of the inhibition of root growth by auxins have already been made (Thimann, 1936; Went and Thimann, 1937). During the past two years a number of further experiments on the inhibition of root growth by auxins have been performed, and some of the results will be presented below.

MATERIALS AND METHODS.—The method used for the determination of the root inhibiting activity of a given substance was essentially that of Lane (1936). Avena grains were soaked for two hours in distilled water and were then allowed to germinate for 24 hours on moist filter paper in the dark at 25°C. At this time when the primary roots were 2-3 mm. long, comparable plants were selected, and the remainder discarded. Each substance to be tested for root inhibiting activity was used in concentrations decreasing by steps of 10 from 10-3 molal to concentrations sufficiently low (10-5 to 10<sup>-11</sup> molal) to be without effect. Four cc. of each solution to be tested were placed in a 10 cm. Petri dish containing a 7 cm. filter paper, and 10-15 of the selected 24-hour-old seedlings were placed in it.

<sup>1</sup> Received for publication May 1, 1939. Published as a report conducted with the aid of the Works Progress Administration, Official Project Number 165-036999, Work Project Number 6330-6989. The authors are deeply indebted to Philip Divirian for his able assistance with this work.

Each solution was always tested in duplicate (2 Petri dishes) and in one experiment 10 or more solutions were usually tested. After the germinating seeds had been placed in the experimental solutions, the dishes were returned to an incubator at 25°C. where they were allowed to remain for exactly 20 hours. At the end of this time they were removed, the length of the longest root of each plant measured, and mean values obtained for the growth in each solution.

This experimental technique gave very reproducible results from day to day. For example, the mean root growth of control plants growing for 20 hours in distilled water in experiments during successive days of February, 1937, were 14.5, 14.0, 14.0, 15.3, 13.9, 15.3, 14.6, 13.4, and 14.3 mm.

The chemical substances tested for root inhibiting activity were those used by Koepfli, Went, and Thimann (1938). Uracilacetic acid, prepared in these laboratories, was also tested.

PRELIMINARY EXPERIMENTS.—In figure 1 is given the relation between molal concentration of indoleacetic acid and root growth during the standard 20-hour experimental period, based on 5 experiments. In a solution of 10<sup>-3</sup> molal indoleacetic acid root growth was almost completely inhibited. With decreasing concentration of the substance, root growth increased, and concentrations below 10<sup>-11</sup> molal caused no measurable effect. No consistent increase of root growth in low concentrations of indoleacetic acid, such as those reported by Amlong (1936), Fiedler (1936), and Geiger-Huber and Burlet (1936), was observed in these experiments. In a number of experiments the lengths of the coleoptiles were also measured, and the results are included in figure 1. The application of indoleacetic acid to the roots of Avena seedlings under these conditions resulted only in decreases of coleoptile elongation, a result in agreement with those of Marmer (1937) and others.

Table 1. Decrease of inhibition of root growth with time in a solution initially 10-7 molal in indoleacetic acid, with simultaneous determination of auxin left in the solution.

Length of experimental period—hours	8	16	20	24	32	50	74
Growth in 10-7 m. indoleacetic acid, mm	0.5	3.1	8.1	14.5	24.0	39.8	73.0
Growth in H <sub>2</sub> O, mm	4.5	13.3	18.9	27.5	39.8	58.0	93.0
Growth, percentage of H <sub>2</sub> O control	11	23	43	53	60	69	78
Growth during period, percentage of H <sub>2</sub> O control Percentage of original indoleacetic acid left in	11	30	89	75	77	87	95
	100	100	39	11	0	0	0

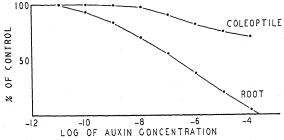


Fig. I. Relation of concentration of indoleacetic acid to inhibition of root and coleoptile growth of Avena seedlings.

If root growth under the above standard conditions be plotted against the log of the concentration of auxin, as is done in figure 1, a close approximation to a linear relation is obtained for root growth inhibitions between 80 and 20 per cent (as compared to control roots in water alone). The concentration of auxin needed to bring about an inhibition of 50 per cent can readily be obtained by interpolation. Thus from figure 1, the concentration of indoleacetic acid which inhibits root growth to just onehalf that obtained in the water controls is approximately  $3\times10^{-7}$  molal. Later in this paper the concentration effective in just causing a 50 per cent inhibition of root growth under these standard conditions will be used as one measure of the relative effectiveness of different auxins in root inhibition.

It was found that the length of the experimental period exerts a profound effect on the effectiveness of indoleacetic acid in causing root inhibition. For example, if a 44-hour experimental period was used, 100 times as high an initial concentration of indoleacetic acid was required to bring about 50 per cent inhibition as was needed to exert the same inhibiting effect over a 20-hour period. During the experimental period, therefore, the solution of indoleacetic acid becomes progressively less effective in inhibiting the growth of roots. This is in part attributable to the disappearance of indoleacetic acid from the solution, presumably due to its destruction by the roots. This was shown by experiments in which the decrease of root inhibition with time was measured and in which the auxin left in the inhibiting solution was simultaneously determined by the Avena test (table 1). However, at least one other factor also enters in e.g., the accumulation of root growth stimulating substances in the medium. This was demonstrated by experiments in which seedling Avena plants were allowed to grow in water or in indoleacetic acid solutions for 20 hours, after which they were transferred to fresh solutions. Fresh plants were then placed in the old solutions—i.e., the solutions in which plants had already grown for 20 hours. By comparison with control plants in fresh water it was found that these "old" solutions actually stimulated root growth by 15 to 30 per cent in different experiments. Both water control solutions and indoleacetic acid solutions which had once supported root growth appeared then to contain something promotive to root growth. This substance, or these substances, might appear to have diffused from the seedling plants during the first

Table 2. Diffusion from seedling plants of substances exerting a promotive effect on the growth of roots. Indoleacetic acid 10-7 molal.

		Growth, per- centage of water controls
Day 1 (1)	or in fresh water for 20	
	hours	50
Day 2 (2)	Fresh plants in fresh I. A. A. <sup>a</sup>	
	or in fresh water for 20	
	hours	45
Day 2 (3)	Plants of (1) left for a further	
	24 hours in same solutions	84
Day 2 (4)	Plants of (1) transferred to	
	fresh solutions for a further	
	24 hours	45
Day 2 (5)	Fresh plants placed in solu-	
, _ (0)	tions of (1) and compared	
	with water controls of (2)	130

<sup>&</sup>lt;sup>a</sup> Indoleacetic acid.

Table 3. Promotive effect of vitamin  $B_1$  on the growth of Avena seedling roots.

Solution used	Root growth in percentage of distilled water controls
Vitamin B <sub>1</sub> , 2 gamma/cc. (optimum conc.	) 127±3.3
Vitamin B <sub>1</sub> , 0.2 gamma/cc Distilled water in which seedling plants ha	
remained for 20 hours	
Fresh distilled water	. 100±1.7

experimental period. The promotive effect of used solutions could not be duplicated by balanced salt solutions, or balanced salt solutions plus sugar and/or various amino acids. It could, however, be duplicated by the addition of yeast extract or of crystalline vitamin  $B_1$  to fresh distilled water, as is shown in table 3. Although no further work has been done on this aspect of the problem, it seems possible that vitamin  $B_1$  or other substances promotive of root growth leach out of the seedling plant during the course of the root inhibition test.

THE RELATION BETWEEN CHEMICAL STRUCTURE AND ROOT INHIBITING ACTIVITY.-It is known that numerous organic acids possess auxin activity in promoting the growth of stems and coleoptiles (Haagen-Smit and Went, 1935; Kögl and Kostermans, 1935; Koepfli, Thimann, and Went, 1938), in the initiation of root primordia on cuttings (Thimann, 1935; Zimmerman and Wilcoxon, 1935), and in other plant growth responses. These organic acids have in common certain structural features which have been termed the "minimal structural requirements" for auxin activity (Koepfli, Thimann, and Went, 1938). The root inhibiting activities of several of these auxin "analogs" have been reported by Lane (1936), Marmer (1937), and others. During the present work, however, a large number of auxin analogs have been tested for root inhibiting activity under closely comparable conditions in order to determine whether or not the minimal structural requirements for other auxin activities hold also for root inhibition.

In table 4 the activities of 21 such analogs of indoleacetic acid are summarized. In the first column the concentration of each substance required to give 50 per cent inhibition of root growth is given. These values were obtained by interpolation as explained above. In the second column the activities are given relative to indoleacetic acid. The activities of these substances in the pea test (Went, 1934) have been determined by Koepfli, Thimann, and Went (1938). Reference to their paper shows that the activities of the different analogs in inhibiting the growth of roots are qualitatively correlated with the activities of the same substances in promoting the growth of pea stems. Thus with increasing length of fatty acid side chain the activity of the indole derivatives or of the phenyl derivatives decreases both in root inhibiting action and in stem growth promoting properties. Phenylacetic acid is less active than indoleacetic acid, and cyclohexaneacetic acid is almost completely inactive in both cases. Naphthalene and anthraceneacetic acid are highly active in both cases. With regard to steric factors also the chemical structures required for the two types of activities seem to be similar, as is shown by the high activity of ciscinnamic acid and the inactivity of trans-cinnamic acid as well as by the inactivity of a, a-dimethyl-

<sup>2</sup> For the sake of simplicity in the presentation, the term auxin "analog" will be used to refer to substances, other than auxins a and b or indoleacetic acid, possessing auxin activity in the *Avena* and/or pea tests.

toluic acid (as compared to phenylacetic or hydratropic acid. It seems justifiable to conclude that the chemical specificity of an auxin in relation to root inhibition is qualitatively closely similar to the chemical specificity of an auxin in relation to stem growth.

There are, however, quantitative differences between the relative activities of these analogs in different tests. This is shown in table 5, in which the

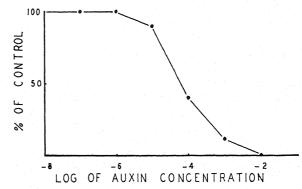


Fig. 2. Relation of concentration of phenylacetic acid to inhibition of root growth of Avena seedlings.

activities of indoleacetic acid and 3 of its analogs are given for the root inhibition test as well as for the Avena and pea tests. In the Avena test the activities are widely separated—e.g., cis-cinnamic acid is 1/1700 as active and phenylacetic acid 1/5000 as active as indoleacetic acid. In the pea test, on the contrary, these activities are much closer together, with cis-cinnamic acid equivalent to indoleacetic acid in activity and with phenylacetic acid only 10 times less active. The relative activities in root inhibition of these various auxin analogs as judged by the concentrations required for 50 per cent inhibition of root growth are intermediate between the relative activities of the same substances in the Avena test and in the pea test. Thus indoleacetic acid is 100 times more active than cis-cinnamic acid and 330 times more active than phenylacetic acid.

A comparison of the concentrations of these analogs needed to bring about a 50 per cent inhibition of root growth is, of course, purely arbitrary. It would also be of interest to compare the concentrations needed to give complete inhibition of root growth. In figure 2 is shown a curve of root growth plotted against molal concentration of inhibiting solution for phenylacetic acid. This curve whose shape is typical of those obtained for all the analogs tested with the exception of a-methyl-3-indoleacetic acid differs from that for indoleacetic acid itself in that (a) the slope of the linear portion is steeper, and (b) it is linear only over a limited range of root inhibitions and tends to approach the concentration axis asymptotically. No explanation for the differences of this curve from that for indoleacetic acid can be offered at present. It might be noted, however, that with the pea test also if growth response is plotted against the log of auxin concentration, the

Table 4. Activities of various analogs of indoleacetic acid in root growth inhibition. Molal concentration needed for 50 per cent inhibition obtained by interpolation (see text).

Name of substance	Structure of substance	Molal concentration needed for inhibition of root growth by 50 per cent	Activity relative to indoleacetic acid for root inhibition of 50 per cen	
3-Indolecarboxylic acid		>10-3	0	
	N H			
3-Indoleacetic acid	$-CH_2COOH$	3×10-7	100	
	Ň H			
3-Indolepropionic acid	$-(CH_2)_2COOH$	2.5×10 <sup>-5</sup>	1.2	
	N H			
3-Indolebutyric acid	—(CH <sub>2</sub> ) <sub>3</sub> СООН	3×10 <sup>-6</sup>	10	
	Ň			
3-Indolevaleric acid	$-(CH_2)_4COOH$	8×10-5	0.4	
	N H			
Phenyl carboxylic acid (benzoic acid)	—СООН	>10-3	0	
Phenyl acetic acid (α-toluic acid)	$-\text{CH}_2\text{COOH}$	1×10-4	0.3	
β-Phenylpropionic acid (hydrocinnamic acid)	(CVI ) COOVI			
	$-(\mathrm{CH_2})_2\mathrm{COOH}$	5×10 <sup>-4</sup>	0.06	
γ-Phenylbutyric acid	$\bigcirc$ $-(\mathrm{CH_2})_3\mathrm{COOH}$	1×10-3	0.03	
δ-Phenylvaleric acid	$-(CH_2)_4COOH$	1×10-3	0.03	
$\gamma$ -Phenylbutyric acid	—CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> COOH	1×10-3	0.03	
a, a-Dimethyl-a-toluic acid	CH <sub>3</sub>			
a, a-Dimeniyra-condic acid	CH <sub>3</sub>	>10-3	0	
3-Indoleacetic acid	$-\text{CH}_2\text{COOH}$	3×10 <sup>-7</sup>	100	
Phenylacetic acid (α-toluic acid)	Н —СН₂СООН	1×10 <sup>-4</sup>	0.3	

Table 4. Concluded.

Name of substance	Structure of substance	Molal concen- tration needed for inhibition of root growth by 50 per cent	Activity relative to indoleacetic acid for root inhibition of 50 per cent
α-Naphthaleneacetic acid	—CH <sub>2</sub> СООН	7×10-6	4
Anthraceneacetic acid	_сн2соон	1×10-6	30
2-Benzofuraneacetic acid	-CH <sub>2</sub> COOH	3×10-4	0.1
Uracil acetic acid	N—C-OH 		
	$ \begin{vmatrix}                                    $	>10-3	0
Cyclohexaneacetic acid	$CH -CH_2COOH$ $H_2C$ $CH_2$ $H_2C$ $CH_2$ $C$ $H_2$	1×10-3	0.03
3-Indolepropionic acid	$-\text{CH}_2\text{CH}_2\text{COOH}$	8×10-5	0.4
α-Methyl-3-indoleacetic acid	CH-COOH	1×10-7	300
Trans-cinnamic acid	С-Н    НС-СООН	>10-3	0
Cis-cinnamic acid	С-Н		
	нооссн	3×10 <sup>-5</sup>	<b>.</b>
β-Phenyl propionic acid (hydrocinnamic acid)	$-$ CH $_2$ CH $_2$ COOH	5×10-4	0.06
Hydratropic acid	СН <sub>3</sub>	3×10 <sup>-4</sup>	0.1
Atropic acid	$CH_2$ $-C$ -COOH	4×10 <sup>-5</sup>	0.8

curve obtained for indoleacetic acid possesses a lesser slope than those of the analogs (Went and Thimann, 1937).

Table 5. Relative activities of analogs of indoleacetic acid in inhibiting root growth. Relative activities for 50 per cent inhibition taken from table 4. Relative activities for 100 per cent (complete) inhibition obtained by extrapolation (see text). Relative activities in Avena and pea tests are included for comparison. Activity of indoleacetic acid taken as 100 per cent in each case.

	Activity relative to indole- acetic acid							
	In root ir	hibition	In					
Substance	By 50%	By 100%	Avena test <sup>a</sup>	In pea test <sup>b</sup>				
Indoleacetic acid	100	100	100	100				
Indolepropionic acid	1.2	40	0.1	100				
Phenylacetic acid	0.3	27	0.02	10				
Cis-cinnamic acid	1.0	100	0.06	100				

<sup>&</sup>lt;sup>a</sup> Activities for Avena test taken from Went and Thimann (1937).

The fact that the curves for the analogs are not linear at high concentrations of analog makes a direct experimental determination of the analog concentration needed for complete inhibition difficult and inaccurate. It is possible, however, that this non-linearity is due to differences in secondary properties of each analog, for example in rate of penetration into the tissue. That such differences do exist has been shown by Went and White (1939). On this assumption, the concentration needed for complete or 100 per cent inhibition has been taken as the intercept on the concentration axis of the extrapolated linear portion of each curve. This has been done for all the substances listed in table 4. The results are of necessity crude, and only 4 of them are tabulated in table 5. The relative concentrations needed for complete inhibition, as obtained in this manner, are, however, strikingly similar. Ciscinnamic acid possesses an activity equal to and phenylacetic acid an activity only 4 times less than that of indoleacetic acid. Eight substances, indoleproprionic, indolebutyric, indolevaleric, a-methyl-3-indoleacetic, naphthaleneacetic, anthraceneacetic, atropic, and cis-cinnamic acids, possessed activities of 40 per cent or more of the activity of indoleacetic acid. Other analogs, however, such as phenylbutyric and phenylvaleric acid, are even by this criterion, 50-100 times less active than indoleacetic acid. The relative activities obtained in this arbitrary manner resemble closely those obtained for the pea test (D. Bonner, 1938).

RELATION OF pH TO ROOT INHIBITION.—It has been shown repeatedly (Dolk and Thimann, 1932; Bonner, 1934; Marmer, 1937; D. Bonner, 1938) that auxins are more effective as growth promoting

substances when present in the unionized state, and that because of this fact the pH of the solution in which the auxin is submitted to the plant exerts an influence on the subsequent growth response. In the pea test or in the Avena cylinder test (Bonner, 1933), for example, indoleacetic acid is much more active in bringing about growth if the solution is buffered at pH 4 than if the solution is buffered at pH 7. Experiments on the relation of pH to root inhibition by auxin were therefore performed for comparison with the earlier results on stem and coleoptile growth. McIlvain's standard buffer solution diluted 8-10 times was used. Preliminary experiments showed that the buffer itself, diluted thus, exerted no effect on root growth in the standard test between pH 4 and pH 7. Table 6 gives the result of one of

Table 6. Relation of pH of external medium to root inhibition by indoleacetic acid solutions.

	Concentration needed to give 50 per cent inhibition
Initial pH 3.80 } Final pH 4.10 }	3×10⁻7 molal
Initial pH 7.00 }	$1\times10^{-5}\mathrm{molal}$

a large number of experiments in which the effectiveness of indoleacetic acid buffered initially at pH 3.8 was compared with the effectiveness of the same auxin solution buffered at pH 7. It may be seen that at pH 7 approximately 30 times as high a concentration of indoleacetic acid was needed to bring about 50 per cent inhibition of root growth as was needed at pH 4. Just as in acid solution indoleacetic acid is more effective in promoting shoot or coleoptile elongation, so also in acid solution indoleacetic acid seems to be more effective in causing root inhibition.

It has been claimed that potassium or sodium salts of indoleacetic acid or other auxins are more effective than are the undissociated acids in the *Avena* test and in other growth tests (Avery, Burkholder, and Creighton, 1937; Zimmerman and Hitchcock, 1935). Although it has already been shown conclusively that this is not the case (D. Bonner, 1937), it is still worth mentioning that in root

Table 7. Relative activities of indoleacetic acid and K indoleacetate in buffered and unbuffered solutions. All substances 10-7 molal.

		Growth in percentage of water controls
D	(Indoleacetic acid	50
Buffered at pH 6.2	··· K indoleacetate	56
TT 1 M 7	(Indoleacetic acid	46
Unbuffered	··· K indoleacetate	48

<sup>&</sup>lt;sup>b</sup> Activities for pea test taken from D. Bonner (1938).

Table 8. Does root inhibition of auxin depend on site of application of the auxin?

		Growth in percentage of water control								
Auxin applied:	Auxin conc. m./ 10-6	10-7	10-8	10-9	10-10	0 (water)				
To entire root and	to endosperm (stand-									
ard procedure)		60	78	96	97	100				
To root tip		53	76	98	100	100				
	9	35	67	97	100	100				

inhibition also, when indoleacetic acid and potassium indoleacetate of equal purity are compared in buffered solutions, their activities are essentially identical. For this purpose freshly recrystallized samples of potassium indoleacetate and indoleacetic acid (from the same lot as that from which the salt was prepared) were used. In table 7 it may be seen that the two substances when made up in equimolar concentrations and buffered at pH 6.2 (the pH of the root) gave essentially identical root inhibitions. If the solutions were unbuffered, the potassium salt was somewhat less active than the acid.

On the nature of root inhibition.—One might next inquire as to whether the inhibition of root growth by auxins has any relation to the distribution or availability to the root of the substances essential to root growth. This problem can be attacked, since the chemical substances essential for the maintenance of root growth are now relatively well known (White, 1934; Bonner, 1937; Addicott and Bonner, 1938; Robbins and Schmidt, 1939). Cooper (1938), Went (1939a), and Stuart (1938) have shown that auxin gradients within the plant may influence the translocation both of other hormones and of sugars, etc. An attempt was therefore first made to discover whether or not the direction of the auxin gradient within the root exerts an influence upon root inhibition. Solutions of indoleacetic acid were applied either to the root tip alone, to the endosperm alone, or to the entire root and endosperm (as in the standard root inhibition test). Root growth was inhibited in all cases, although auxin applied at the base of the root was somewhat more effective than auxin applied at the root tip alone (table 8). It would seem unlikely, however, that the direction of auxin flow within the root is of any considerable importance in root inhibition.

Experiments of a different nature were designed to find out whether roots inhibited by auxin contained more or less of the materials needed for root growth than roots not inhibited by auxin. Root inhibition tests were carried out in the usual manner, using 10<sup>-7</sup> molal indoleacetic acid. After the 20 hours of the test 5 mm. tips were cut both from inhibited roots and from control roots, and these tips were placed in the solutions indicated in table 9. It may be seen from table 9 that in water alone neither the inhibited nor the control tips subsequently grew to any appreciable extent. In a balanced root solution (that used for the culture of isolated pea roots, Bonner and Addicott, 1937), however, the growth was more extensive and was somewhat larger in the case of the tips from roots previously inhibited by indoleacetic acid. The addition of sucrose to the mineral salt solution did not increase the final length of the roots but did increase the rapidity with which this final length was attained. Addition of vitamin B<sub>1</sub> to the medium resulted in a further increase of 50 per cent in the final length. In this case also the tips from inhibited roots grew only slightly more than the tips from non-inhibited roots. It seems necessary to conclude, therefore, that the materials essential for root growth are neither (a) accumulated in great excess in the extreme tips of inhibited roots nor (b) completely prevented from reaching the tips of inhibited roots. Similar experiments in which the whole root rather than a 5 mm. tip was excised after inhibition by auxin showed that the materials for root growth

Table 9. Growth of excised Avena root tips after treatment of the seedlings with or without indoleacetic acid. Seedlings placed in indoleacetic acid, 10-7 molal, or water for 20 hours.

		Growth in mm. after				
5 mm. tips placed in	Tips from	1 day	2 days	3 days	5 days	
Water	$\cdots$ $H_2O$ I. A. A.	1.7 1.7	2.0 1.7	1.9 1.8	2.0 1.9	
Nutrient salt solution	$\cdots$ $H_2O$ $I. A. A.$	1.9 1.8	4.3 4.2	5.6 7.0	7.0 8.6	
Nutrient + sucrose 2 per cent	$\cdots$ $\left\{ \begin{array}{l} H_2O \\ I, A, A. \end{array} \right.$	4.6 5.2	6.5 $7.4$	6.6 7.7	7.0 8.2	
Nutrient + sucrose + B <sub>1</sub>	$\cdots egin{cases} \mathbf{H_2O} \\ \mathbf{I.A.A.} \end{cases}$	5.0 5.8	7.6 8.5	9.3 10.3	11.0 12.8	

do not accumulate in the base of the root during root inhibition.

In further experiments, root inhibition tests were carried out with Avena in the usual manner, but various other substances were added to the inhibiting medium. Table 10 shows that if vitamin  $B_1$  was

Table 10. Root inhibitions by auxin in the presence of vitamin B<sub>1</sub> and/or of sucrose in the medium. Indoleacetic acid 10<sup>-6</sup> molal in all cases.

Growth in percentage of water controls in:						
Indoleacetic acid alone	$32 \pm 0.7$					
Indoleacetic acid and vitamin B <sub>1</sub> 20 gamma/cc.	$49 \pm 2.7$					
Indoleacetic acid and sucrose 2 per cent	$45 \pm 0.9$					
Indoleacetic acid, sucrose and vitamin $B_1$	$51 \pm 1.6$					

added to the medium, the roots in auxin were inhibited somewhat less than if vitamin  $B_1$  was not added. A similar effect was brought about by 2 per cent sucrose. Other substances such as mineral salts, various amino acids, etc., were without effect. Thus, although root growth in the presence of indoleacetic acid may be limited to a slight extent by the available vitamin  $B_1$  and the available sugar, still the effect is not a major one, and it seems justifiable to conclude that the inhibition of root growth by auxins is not associated primarily with a deficit in any of these root growth factors.

A strong indication that the inhibition of root growth by auxin is attributable to a direct effect of the auxin rather than to an effect on the distribution of other necessary growth factors involved in root growth was also obtained by experiments with excised pea roots. The first experiments were carried out with root tips freshly excised from seedling plants and cultured aseptically with the technique and medium described elsewhere (Bonner and Addicott, 1937). It has been shown earlier that the freshly excised pea root tip contains an ample supply of vitamin B<sub>1</sub> and of nicotinic acid and hence is not limited in its growth by these substances. Such tips grow approximately 60 mm. during one week if supplied only sucrose and the requisite inorganic salts. In table 11 it is shown that the growth of these excised roots is inhibited by added indoleacetic acid just as is the growth of roots attached to the seedling plant. The addition of vitamin  $B_1$  to the culture medium exerted no effect on the growth of the control (without added indoleacetic acid) roots and but little effect on the inhibition by added indoleacetic acid. In these series a slight stimulation of root growth was found with auxin concentrations between  $10^{-8}$  and  $10^{-10}$  molal, in contrast to the results presented above for Avena roots, but in agreement with the finding of Fiedler (1936) and of Geiger-Huber and Burlet (1936) for isolated corn roots.

In other experiments isolated pea roots which had been one week in culture were subcultured (by removal of 10 mm. tips) into medium containing as accessory growth factors both vitamin  $B_1$  and nicotinic acid. It is known (Addicott and Bonner, 1938) that this combination of growth factors will allow unlimited rapid growth of the pea root, and it may be inferred, therefore, that such a medium supplies all the substances required for the growth of the pea root. Even in this medium, however, inhibition of root growth by applied auxin was readily obtained, 50 per cent inhibition of root growth being given by a solution approximately  $10^{-7}$  molal in indoleacetic acid.

It is known (Went, 1939b) that a pretreatment of pea stems with certain substances (themselves inactive in the pea test) greatly increases the sensitivity of these stems to subsequent treatment with indoleacetic acid. Thus pretreatment for 2 hours with gamma-phenylbutyric acid or cyclohexaneacetic acid may increase by as much as 100 times the subsequent curvature given by split pea stems immersed in indoleacetic acid of low concentration. Potassium anthraquinone sulfonate and sodium naphthol sulfonate pretreatments have been shown to increase the number of root primordia formed by cuttings in response to treatment with indoleacetic acid (Went, 1939a). A similar type of experiment was therefore carried out to determine whether or not the above substances also "facilitate" the action of indoleacetic acid in root inhibition. Avena seeds were germinated for 24 hours in the standard man-

Table 11. Inhibition by indoleacetic acid of the growth of isolated pea roots grown under sterile conditions in vitro. Mean of 4 experiments each with 20 roots at each concentration in each series.

Indoleacetic acid.	Growth in percentage of controls with no added I. A. A.								
Concentrate molal	10-3	10-4	10-5	10-6	10-7	10-8	10-9	10-10	Control
Growth in the absence of added vitamin B <sub>1</sub> . Percentage of controls with									
no added I. A. A	4	22±1	49±1	66±2	89±2	115±3	110±2	110±2	100±2
centage of controls with no added I. A. A		21±1	27±3	68±2	80±2	115±2	105±2	105±2	100±2

ner, and they were then immersed for 2 hours either in water or in solutions (100 mgs. per liter) of phenylbutyric acid, potassium anthraquinone sulfonate, or sodium napthol sulfonate. After this 2-hour pretreatment the seedlings of each treatment were distributed into Petri dishes containing either water or indoleacetic acid in concentrations ranging from  $10^{-4}$  to  $10^{-10}$  molal. None of the pretreatments exerted any significant effect either on the subsequent growth of the roots in water or on their sensitivity to inhibition by indoleacetic acid. The action of auxins in root inhibition therefore differs from their action in the pea test or in root initiation in that the root inhibiting reaction is not facilitated by pretreatment with the above substances.

GENERAL DISCUSSION .- Auxins within suitable concentration ranges promote and are essential to the growth of many stems, petioles, the coleoptiles of grasses, etc. On the other hand, in similar concentration ranges they inhibit the growth of roots. These two seemingly diverse manifestations of physiological action are apparently both fundamental attributes of auxin activity. Thus among the analogs of indoleacetic acid those substances which possess great activity in promoting the growth of the Avena coleoptile possess great activity also in inhibiting the growth of roots. All the analogs active in the pea test are active (in the same order of decreasing activities) in root inhibition, and those analogs which are inactive in the pea test are also inactive in root inhibition. This means then that the chemical structure which a molecule must possess in order to exert growth activity on stem or coleoptile growth is closely similar to that which it must possess in order to be effective in auxin root inhibition. Increase in external pH also decreases the activity of auxin as a root inhibitor just as it decreases the activity of auxin in promoting the growth of stems or of coleoptiles.

These facts suggest strongly that auxin enters into an initial reaction which is common to root inhibition and to shoot elongation. This is essentially the hypothesis of Thimann (1935) who has suggested that auxin enters into some "master reaction" and that other properties of the particular tissue then determine what sort of an effect will be manifested.

It has been suggested above that the concentrations of auxin analogs needed to give complete inhibition of root growth may not be greatly different, at least in the cases of 9 of the more active substances. This might suggest that there is a definite number of reactive groups within the root and that if all of these are combined with an auxin, complete inhibition of root growth ensues. That is, the inhibition of root growth may depend on a stoichometric

relation between auxin and reacting tissue, just as has been shown to be the case (D. Bonner, 1938) in the response of pea stems to auxins.

Since the various nutritional factors needed for root growth are relatively well understood, an attempt has been made to offset auxin inhibition by a superabundance of the material needed for root development. This was possible, however, only to a very limited extent with the Avena root and impossible with isolated pea roots. Root growth was inhibited by auxin even in the presence of adequate supplies of all of the materials needed for growth of the pea root. It seems highly unlikely, therefore, that auxin inhibition of root growth is attributable to any kind of direct effect upon the availability of accessory root growth factors. Rather, it would seem necessary to assume that auxin acts directly on the root in some as yet obscure manner.

#### SUMMARY

The activities in inhibiting the growth of Avena seedling roots of 21 analogs of indoleacetic acid have been compared as to the concentrations needed to give 50 per cent inhibition of root growth. It is shown that the chemical specificity of the auxin inhibition of root growth is closely similar to the chemical specificity of auxin promotion of stem and coleoptile growth, since those substances such as indoleacetic acid, naphthaleneacetic acid, anthraceneacetic acid, phenylacetic acid, and cis-cinnamic acid which are relatively highly active in the Avena or pea test are also highly active in inhibiting root growth, while those substances such as cyclohexaneacetic and trans-cinnamic acid which are little active in promoting stem growth are also little active in inhibiting root growth. The relative activities in root inhibition of the 21 auxin analogs studied are similar to, but not identical with, relative activities of the same substances on stem growth.

Indoleacetic acid is more effective in inhibiting root growth in acid (pH 4) media than in media of higher alkalinity. The relation of pH to auxin activity resembles thus the relation known to exist between pH and auxin promotion of stem growth.

It is shown that the inhibiting effect of auxin on root growth cannot be offset to any significant effect by supplying the root with large excesses of the materials needed for root growth. The effect of auxin in root inhibition seems thus to be directly upon the root cells rather than upon the mobilization or transport of other growth substances.

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# THE CAUSES OF SELF-STERILITY IN RYE 1

Margaret Landes

It is well known that rye is partially self-sterile (Brewbaker, 1926; Peterson, 1934; and others). While there is considerable variability, the average seed setting in the first year of selfing is under 10 per cent, but by selection individual plants which are highly self-fertile may be obtained (Peterson. 1934; Krasniuk, 1935). Following the work of East (1929) on Nicotiana, self-sterility in general has been regarded as due to the failure of pollen-tube growth through the action of a series of multiple alleles. Peterson (1934) interpreted data collected in an attempt to improve rye through selection of highly self-fertile lines in accordance with the general conceptions of East. Sears (1937) concluded that the general conception of self-sterility through the failure of pollen-tube growth is applicable to rye, since he found that few of the grains become empty. Nevertheless, Boyes and Thompson (1937), in connection with a study carried on for another purpose, found evidence of abnormalities in the development of the endosperm of rye which might be responsible for failure of seed setting after selfing. Many attempts have been made to obtain highly selffertile lines of rye, but none of these has been very successful. A number of these studies have been reviewed by Stout (1938).

The present study was undertaken to determine the frequency with which fertilization may occur in rye after self-pollination and whether or not selfsterility might be due to abnormal processes subse-

quent to fertilization.

METHODS.—Selfed ovaries of Prolific rye were fixed at various periods from 1 to 14 days after pollination. They were embedded, sectioned, and stained in the usual way. For comparison, ovaries of the same plants which had been crossed with quite unrelated plants of another variety were treated in the same way as the selfed ones. In addition, plants selected at random where pollination was open were harvested and examined for seed setting. Rye pollen from a number of plants was examined.

NORMAL DEVELOPMENT OF EMBRYO AND ENDO-SPERM OF RYE.—In the embryo at one day, two to four cells may be present. The endosperm develops rapidly, and thirty to forty free nuclei are present in the cytoplasm in the periphery of the embryo sac. Fifteen to twenty antipodal cells are present on the

ventral side (fig. 1).

By three days, the embryo is 6- to 10-celled and shaped like a pendant. Many free endosperm nuclei are present in the cytoplasm of the embryo sac lining and pocket (fig. 2).

There is considerable increase in the size of the sac at each stage examined. However, growth is most

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This problem was undertaken at the suggestion of Dr. W. P. Thompson, to whom I am greatly indebted for advice and constant assistance throughout the investigation.

rapid from three to nine days, for the embryo sac not only increases greatly in size but the endosperm becomes completely cellular. By five days, the embryo is 16- to 20-celled, and walls are forming in the endosperm of the pocket (fig. 3).

By seven days, the embryo is six or seven cells across and is about twice as long. The endosperm has increased greatly in size and is largely cellular except for areas at the center and antipodal side. Starch grains make their appearance on the ventral side toward the chalazal end. The antipodals have

disappeared.

A nine-day embryo is much like that at seven days except that it is larger and differentiation is beginning. The endosperm is completely cellular. The starch grains have increased in size and extend over a large area. The aleurone layer is clearly distin-

guishable.

At fourteen days the embryo is well advanced. Foliage leaves are differentiated; the coleoptile almost reaches around the growing point; epiblast and root tip are present, and the scutellum is elongated. In the endosperm, the cells on the ventral side are comparatively well filled with starch. On the dorsal side, the starch grains have crowded the nuclei to such an extent that they appear as dense irregular bodies. There is still an area near the embryo in which only a few small grains are present. The cells of the aleurone layer are well differentiated and well filled with aleurone grains. Part of the nucellus is still present.

Measurements were made of the embryo and endosperm at various stages of development, but rye varies so much in size from plant to plant that the results are considered insufficient to be of any value.

Development in selfed ovules and comparison with crossed.—Both selfed and crossed ovaries fall into four groups. In a small percentage, no embryo sac has been formed. In some of these there is a group of small cells at the center of the nucellus, and in others (fig. 4) a narrow strip of deeply staining material extends from the center toward the micropylar end. Presumably the breakdown occurs at or immediately after meiosis. At any rate, in these ovaries no megaspore enlarges to form an embryo sac. This form of breakdown appears to be widespread in rye, having been observed in 9 of 12 plants examined in two different years. It is, of course, not connected with selfing.

In the second group of ovaries, the embryo sac appears to be normal in every respect but remains unfertilized. At five days after pollination signs of degeneration begin to appear. Such ovaries are designated in the tables by the term "unfertilized."

In the third group the embryo sac is apparently formed but soon aborts. The cytoplasm becomes strongly granular and the antipodal nuclei small and

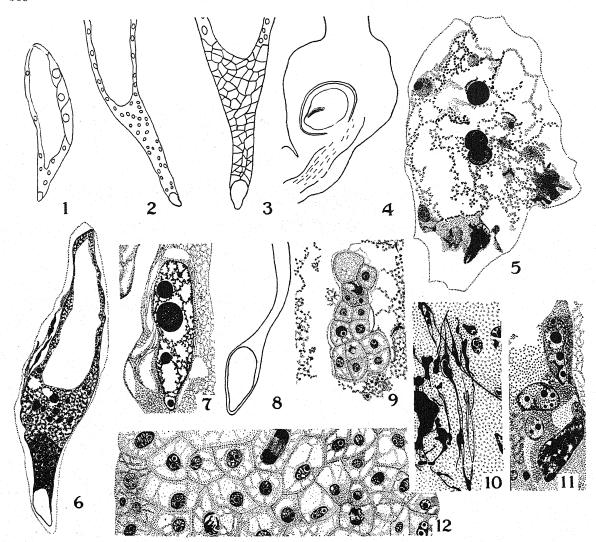


Fig. 1-12.—Fig. 1. Normal 1 da. embryo sac; embryo, free nuclei in the cytoplasm of the endosperm, three antipodal cells at the right.—Fig. 2. Normal 3 da. embryo sac; embryo and part of endosperm.—Fig. 3. Normal 5 da. embryo sac; embryo and part of endosperm.—Fig. 4. No embryo sac formed, deeply staining material extending from center of nucellus toward micropylar end.—Fig. 5. One da. aborted embryo sac showing granular cytoplasm and remains of antipodal cells.—Fig. 6. Abnormal crossed embryo sac; embryo, dense nuclei, and granular cytoplasm of endosperm, remains of antipodal cells at left.—Fig. 7. "Torpedo" shaped nucleus from the endosperm of a selfed 7 da. embryo sac.—Fig. 8. Abnormal crossed 7 da. embryo sac, embryo but no endosperm developed.—Fig. 9. A normal cell from the endosperm of a crossed 14 da. embryo sac.—Fig. 10. Stringy chromatin from endosperm.—Fig. 11. Abnormal nuclei from endosperm.—Fig. 12. Normal strip through endosperm of crossed 7 da. embryo sac.—Fig. 1, 2, 3, 4, 8, ×85. Fig. 5, 6, 7, 9, 10, 11, 12, ×350.

dense or represented by stringy masses of chromatin. Even ovaries collected at one day have aborted to such an extent that only the degenerating antipodal nuclei can be recognized (fig. 5). After seven days the sac may be represented by a large empty space, or the whole ovary may collapse. Comparison of measurements of the aborted sacs with those which had not been fertilized shows that there was no appreciable difference in size.

In the last group some degree of development occurs. In many cases irregularities are found, but in all, development has at least proceeded beyond the normal three-day stage. Many of these developing ovules in which the irregularities are extreme may abort at later stages. These abnormalities affect the embryo less than the endosperm. They include patches of granular cytoplasm which may be large or small, very large or very small nuclei, nuclei with an unusually large number of nucleoli, stringy chromatin in varying amounts, failure of endosperm to fill the space formed by the expanded integuments, and cells with more than one nucleus.

The percentages of ovules of each of these four types (without embryo sacs, unfertilized, aborting

and developing) in selfed and crossed material for 1936 and 1937 are shown in table 1.

TABLE 1.

	Selfed				Crossed			
	1936		1937		1936		1937	
	%	No.	%	No.	%	No.	%	No.
Without embryo sac.	11	5	7	10	10	. 5	7	14
Unfertilized			47	68	17	9	13	26
Aborting	36	17	38	55	17	9	21	41
Developing	49	23	8	12	56	29	59	116
Total		47	4 .	145	4	52		197

The percentage of each of the four types of ovules is very similar in the crossed material in the two years, but in the selfed rve there is a marked difference in the second and fourth classes. In 1936 only 4 per cent of the ovules remained unfertilized, while failure occurred in 47 per cent of the ovules the following year. It is difficult to account for this difference, unless bud pollination occurred as described by East and Mangelsdorf (1926).

In comparing the selfed and crossed rye, the percentage of ovules failing to form embryo sacs in each of the two years is practically the same, as is, of course, to be expected. However, differences of varving degrees are present in the other classes. Only 4 per cent of the ovules selfed in 1936 remained unfertilized in comparison with 17 per cent in the crossed. At the same time, the percentage of developing ovules is relatively high in the selfed rye, being only 7 per cent less than the crossed. However, in 1937, the situation was reversed, and 47 per cent of the selfed ovules were unfertilized in contrast with 13 per cent of the crossed. In comparison with 59 per cent of developing ovules in the crossed rye, there are only 8 per cent in the selfed. The differences in the percentage of fertilized ovules in 1937 were apparently due to differences in pollen tube growth.

The most significant difference between selfed and crossed rye is the fact that the percentage of aborted ovules for both years is relatively twice as great in the selfed rye as in the crossed. While a certain amount of abortion in this group may be due to the same cause as the absence of embryo sacs, this will not account for the much greater degree which is present in the selfed rye. Abortion has in all cases progressed too far to determine whether the pollen tube had entered or fertilization had occurred. But presence of pollen tubes does not necessarily ensure fertilization, for Cooper, Brink, and Albrecht (1937) observed that certain well developed alfalfa ovules remained unfertilized even though pollen tubes were as numerous as in those which began development. At the same time, the higher incidence of abortion in the selfed rye sacs, than in the crossed indicates a relationship between the abortion and the entrance of the pollen tube.

A comparison of the developing selfed and crossed ovaries offers support for this view. While wide deviation from normal development occurs in both selfed and crossed rve, the frequency is higher in the selfed than in the crossed. Since irregularities in embryo and endosperm development are more marked at five days and later when growth is most rapid, no ovaries collected earlier than this are considered here. The numbers of developing ovules from five to fourteen days in 1936 were 8 selfed and 17 crossed, in 1937, 12 selfed and 101 crossed. Of these, 50 per cent of the selfed ovules (1936) and 16 per cent in 1937 were very abnormal in comparison with per cent and 9 percent of the crossed ovules.

It is interesting to note that the greatest number of these irregularities in the selfed rye is found in the 1936 group where the percentage of selfed ovules was highest (table 1), though there are none in the ovules from the same plants which were crossed. In 1937 when there were fewer developing selfed ovules, there are fewer irregularities. The same is

true to a lesser degree of the crossed rye.

The ovules included in this very abnormal group differ in the degree and type of irregularity present. In two seven-day selfed ovules and in one seven-day and one six-day crossed, the endosperm is largely confined to granular cytoplasm which lines the sac and fills the pocket. In the best sac, walls have formed in the lower part of the pocket (fig. 6). A few nuclei which are extremely dense in the crossed ovules, very large and "torpedo" shaped (fig. 7) in one of the selfed ovules and normal in the other, appear in the cytoplasm. In one selfed ovule the embryo is six-celled, but in the six-day crossed ovule it is many-celled. The embryo was not observed in either of the others. In one crossed seven-day ovule, the embryo is approximately twenty-celled, but the endosperm has aborted completely (fig. 8). In a nine-day selfed ovule there is a similar condition though there has been less growth, since the embryo is few celled. All that remains of the endosperm is a small patch of cytoplasm. A condition somewhat similar to this occurred where the sac increased greatly in size but collapsed at the center, leaving a small normal area at the micropylar and chalazal ends, with only cytoplasm between. This was observed in one selfed ovule and two crossed ones. There is still another type of abnormality which is less extreme than those described. The sac is larger and contains much cytoplasm of a granular nature. In it are scattered free nuclei and "islands" of normal cells (fig. 9). There are two selfed and four crossed ovules of this kind.

Irregularities of a lesser kind occur in most of the embryo sacs. These include areas of granular cytoplasm, stringy chromatin (fig. 10), which is usually found near the embryo, nuclei of unusually large size some of which contain seven or more nucleoli (fig. 11), and very small dense nuclei. In a few ovaries the endosperm fails to fill the expanded integuments. More than one kind of abnormality may be present in an embryo sac. A few ovules are free from any irregularity. In 1936, 25 per cent of the selfed ovules at five days and later were perfectly normal in comparison with 88 per cent of the crossed, and in 1937 none of the selfed were normal, though there were 28 per cent in the crossed ovules. A strip through the endosperm of a well developed seven-day crossed ovule is shown in figure 12.

Thus the irregularities which are present in selfed and crossed rye are alike in their nature and differ merely in the frequency of their occurrence. Whether the failure of formation of embryo sacs, the very early abortion of sacs, and the disturbances in endosperm development are due to the same cause is not known. But whatever the cause is, its effect is increased with selfing. In less than one-half of the aborted ovules there is stringy chromatin and other signs of abortion outside the ovule, but this seems to follow rather than cause the abortion of the sac. This is similar to the situation found by Cooper, Brink, and Albrecht (1937).

An examination of twenty-seven spikes from six plants selected at random from the same plot as those plants used in selfing and crossing in 1937 showed that under open pollination there was 54 per cent "seed" setting as compared with 59 per cent developing ovaries in the crossed rye. The percentage of seed setting under conditions of open pollination is somewhat lower than that observed by Krasniuk at Saratov who found an average of 58 per cent during the two-year period 1932-34. It is also lower than that found by Leith (1925) who reported one-third blind florets during a three-year test.

Among the "seeds" are a number which are abnormal in many ways. Some have ceased development at a very early stage, while others have varying degrees of endosperm irregularity which may be correlated with conditions found in the selfed and crossed rye embryo sacs. Some seeds have small shrunken areas which occur in the same region as non-cellular patches in the endosperm. Others have a shrunken constricted area at the center which corresponds to the embryo sacs which have normal areas at either end but collapse at the center. Only a few of the seeds are perfectly smooth.

In 1936, one crossed rye plant set 68 per cent seed, while two selfed rye plants averaged 27 per cent. The seed from the selfed plants was as good as that from the crossed.

Blind florets in rye are apparently due to different causes. While failure of pollen tube growth and con-

sequent failure of fertilization is in part responsible, embryo sacs fail to develop in a small percentage of them. Still others abort at an early stage after the formation of the embryo sac. Both Krasniuk (1935) and Peterson (1934) report that highly self fertilized lines give the least loss of seed setting under field conditions.

An examination of pollen from fifteen plants gave an average of 62 per cent good grains with a range from 1 to 90 per cent. Since rye plants with 14, 15, or 16 chromosomes may be present (Hasegawa, 1934), it was thought that this might be responsible in part for the various abnormalities present in the embryo sacs and pollen grains. However, an examination of the chromosome condition in nine plants showed insufficient irregularity to account for this, though two of the nine had 8 chromosome pairs present.

#### SUMMARY

Rye plants are partially self sterile, seeds developing on less than 10 per cent of the selfed florets.

Embryo sacs of ovaries collected at several dates after self pollination were examined, as were those of cross pollinated ovaries. In a considerable proportion of cases fertilization occurred following selfing so that much of the self sterility cannot be attributed to failure of pollen tube growth but must be due to events subsequent to penetration of the embryo sac by the tube. Failure of pollen tube growth plays some part, because a larger number of ovules were fertilized after cross than after self pollination.

A large proportion of self pollinated ovaries abort in early stages. In these cases no cellular endospermal tissue is formed. This accounts for much of the self sterility. There is also abortion in some crossed ovaries but far less than in selfed ones.

In a proportion of ovules which develop, there are irregularities in the endosperm. These irregularities are more numerous in selfed than in crossed ovules.

In from 5 to 10 per cent of the ovules on most plants examined no embryo sac had formed. The cause of this has not been determined but is probably the same as the cause of the considerable percentage of aborted pollen in most plants.

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# THE COMPARATIVE HISTOLOGY OF SOME OF THE LAMINARIALES 1

Arlo I. Smith

SINCE MUCH of the previous work on the histology of this group of brown algae has led to considerable contradiction and confusion, this paper is an attempt to present the results of a comparative study of several members of the Laminariales. In its genesis this problem was also meant to deal with the developmental nature of the plant tissues, but upon the promise of its assuming gigantic proportions it has been limited almost entirely to the adult tissues as they exist, without considering how the elements making up those tissues became what they are. Chief among the contradictory questions in existence are those relative to the so-called sieve tubes. What are the sieve tubes in the Laminariales and how do they differ from those of the Spermatophytes?

So far as the author has been able to find there are in existence no photomicrographs of the detailed structure of any of the Laminariales; however, Sykes (1908) did present six photomicrographs of Macrocystis pyrifera and Laminaria saccharina stipes in cross section taken at very low magnifications.

"Trumpet hyphae," or filaments made up of elongate cells with fluted ends (fig. 16), were first discovered in Laminaria saccharina and Alaria esculenta by Reinke (1876) and described as "lange Parenchymzellen mit aufgeblasenen Enden." Wille (1885) later more accurately described and figured them. Will (1884) figured and described trumpet hyphae in Macrocystis and Nereocystis. In his work on obliteration of sieve tubes in Laminarieae, Oliver (1887) reports sieve tubes in Laminaria, Alaria, Agarum, Thallassiophyllum, Ecklonia, Lessonia, Macrocystis, and Nereocystis. In addition to the trumpet hyphae, the last two genera have certain thick-walled perforated cells which he terms "true sieve tubes" resembling sieve tubes of Cucurbita of the squash family.

Humphrey (1887), in working on Agarum Turneri, corroborates Oliver's report of trumpet hyphae in that genus. Rosenthal (1890) traced the development of young sieve tubes in Macrocystis and Thallassiophyllum. Results reported a year later by

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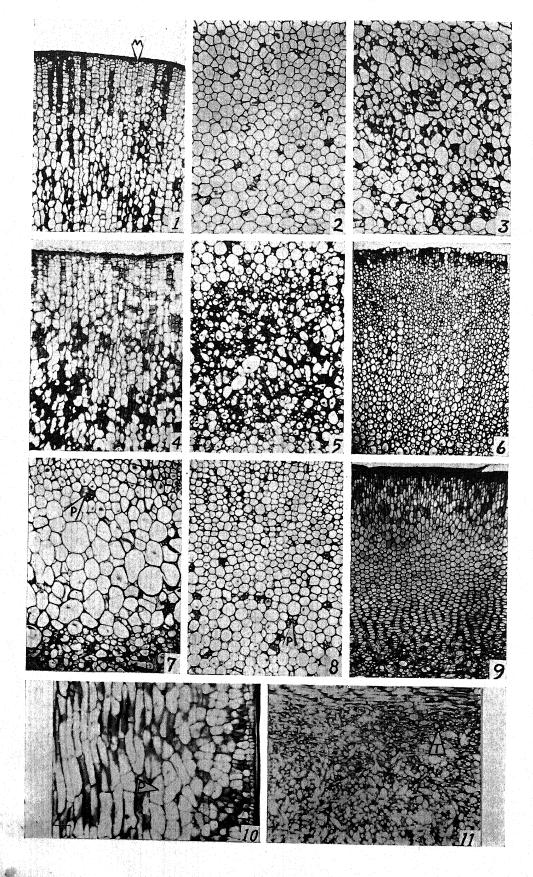
Setchell (1891) on Sacchoriza (Laminaria) dermatodea compare favorably with Rosenthal's (1890) conclusions on sieve tube development in Macrocystis. Trumpet hyphae are poorly developed in Laminaria bulbosa according to Barber (1889). Wille (1897) considers the trumpet hyphae to be homologous with the large sieve tubes reported for Macrocystis, while Oliver (1887) maintains that they are of different nature. It seems fairly agreed that the large tubes in the medulla of Macrocystis and Nereocystis are true sieve tubes (Oltmanns, 1904; Oliver, 1887; Rosenthal, 1890; Sykes, 1908; Will, 1884; Wille, 1897), but the question is whether or not the trumpet hyphae should be called sieve tubes. Oltmanns (1904) also questions the use of the term trumpet hyphae. The author does not like the use of the term trumpet hyphae and, instead, in the remainder of the paper proposes to call them trumpet filaments. The term filament seems to be desired rather than hyphae because the former seems to be more nearly related to algae than the term hyphae. Kibbe (1915) reports trumpet filaments, which she also terms sieve tubes, present in Alaria fistulosa.

MATERIALS AND METHODS.—Algae examined for this problem include Laminaria saccharina (L.) Lamouroux, Cymathere triplicata (Post. and Rupr.) J. Agardh, and Costaria costata (Turn.) Saunders of the Laminariaceae, Nereocystis Luetkeana (Mert.) Post. and Rupr. of the Lessoniaceae, and Pterygophora californica Rupr., and Alaria tenuifolia Setchell of the Alariaceae. Classification follows Setchell and Gardner.

The plants examined were of various ages and sizes and were collected both in spring and fall in Puget Sound and the Straits of Juan de Fuca and were preserved directly, either in 7 percent formalin in sea water, or in strong chromoacetic acid in 50 per cent sea water. Fresh material was used only as a check on preserved material.

Sections were made both by the freezing sliding microtome and by the paraffin method. Material to be sectioned in paraffin was previously dehydrated and infiltrated by the butyl alcohol method. Both longitudinal and transverse sections were made from the blade, the transition region between the blade and stipe, the stipe, and from the hapteres. Sections were cut 15 to 20 microns thick on the sliding microtome and I to 10 microns on the rotary microtome.

Microchemical tests were made largely upon sections preserved in formalin or fixed in strong chromoacetic acid solution and run up into 70 per cent alco-



hol (50 per cent ethyl and 20 per cent n-butyl) to which was added a small quantity of glycerine. Microchemical tests included use of iodine-potassium iodide, chlorozine iodide, concentrated sulphuric acid, concentrated chromic acid, concentrated calcium chloride, cuprammonia, and corralin soda.

Sections were stained in methylene blue, aniline blue, corralin soda, fast green, gentian violet, eosin, or safranin. The safranin proved the best general stain, and the pit membranes were more plainly visible in this than in the other stains tried.

Sections were studied under magnifications up to 1800 diameters, with the aid of a  $20\times$  ocular and oil immersion objective.

EXTERNAL MORPHOLOGY.—In the Laminariales the entire plant or frond is usually divided into three distinct regions—viz., a flattened blade or blades, a cylindrical or more or less flattened stipe either simple, dichotomous or irregularly branched, and a holdfast varying from solid discoid to clusters of simple or branched hapteres.

In Laminaria saccharina is found a stipe of variable length but usually around 10 to 20 centimeters. The stipe is cylindric below and flattens above into the transition of blade and stipe. The blade is membranaceous to coriaceous and has an undulate margin. The numerous hapteres are dichotomously branched.

Cymathere triplicata has a small discoid holdfast rather than one of hapteres. The stipe is short, stout and solid, cylindric below and flattened above into the transition region. The linear undivided blade is thick and leathery at its base, becoming thinner at

All photomicrographs were made with a Leitz "Makam" Mikro-Aufsatzkamera, taken on Commercial Ortho Nitrate film. No retouching has been done. Unless otherwise stated, the figures were stained in safranin and the photomicrographs made without the use of filters.

the tip. Three diagnostic longitudinal folds extend the length of the blade.

A typical specimen of Costaria costata has a 5-costate blade which tapers quite rapidly into the flat transition region. The stipe is usually short but may be quite variable in length. Hapteres are dichotomously branched and usually form an extensive hold-fast.

In Pterygophora californica is found a stipe which is comparatively large and has concentric growth rings when viewed in cross section. The stipe is terete below but flattens above in the transition region. From along the margins of this transition region sporophylls arise, there often being 10 to 20 on the plant. A terminal blade is also present, continuing from the transition region, and is frequently hardly larger than the sporophylls.

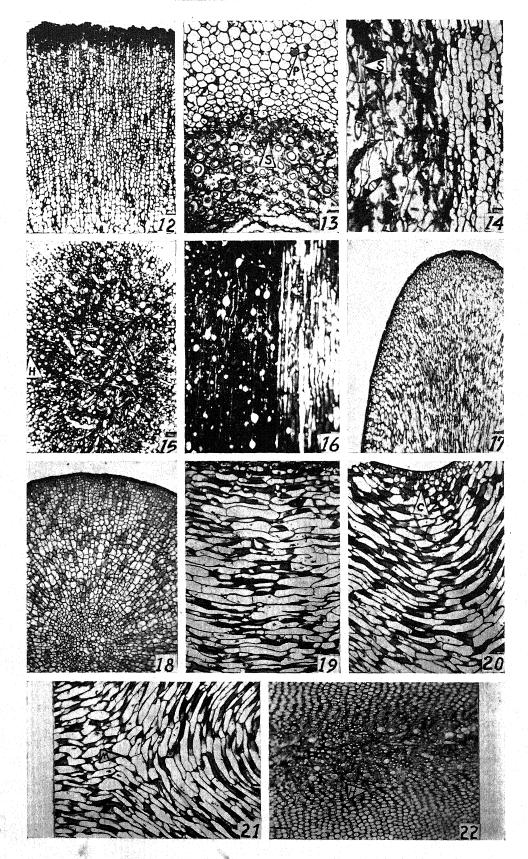
Alaria tenuifolia has a comparatively short stipe which is solid and unbranched and at its upper end flattens into the transition region, whence continues a distinct percurrent midrib through the blade. Sporophylls may be present along the upper part of the stipe. The holdfast is not large and is made up of slender dichotomously branched hapteres.

Nereocystis Luetheana has a very long stipe which is bulbous at its upper end and from which several blades arise in either two or four sets. The transition region is in the pneumatocyst and bases of the blades. The stipe is hollow throughout most of its length, being solid near its base. Hapteres branch dichotomously to form a massive interwoven holdfast.

In the following comparisons the stipe is treated first because its structures make for better orientation.

THE STIPE.—In all cases, whether or not the stipe is solid, there are three definite regions to be observed in both cross and longitudinal sections. The outer cells for several layers deep are definitely of

PLATE I.—Fig. 1-11.—Fig. 1. Costaria costata, cross section of outer cortex of the stipe showing radial rows of cells and the epidermal meristem (M). Compare with figure 4. Section 8 microns thick. ×55.—Fig. 2. C. costata, cross section of inner cortex of the stipe showing cessation of radial arrangement of cells. A few end walls are present in which pits (P) are arranged in a circle. The medulla is located on the lower side of the figure. Compare with figure 42 of plate IV. Section 8 microns thick. ×55.—Fig. 3. C. costata, cross section of medulla of the stipe showing presence of isodiametric cells of varying sizes. Section 8 microns thick. ×55.—Fig. 4. C. costata, longitudinal section of outer cortex of the stipe showing radial arrangement of cells and the epidermal meristem. Compare with figure 1. Section 8 microns thick. ×55.—Fig. 5. Alaria tenuifolia, cross section of the medulla of the stipe showing varying sizes of the isodiametric cells. A few of the smallest cells are trumpet filaments. Compare with figures 8 and 12. Section 10 microns thick. ×55.—Fig. 6. Cymathere triplicata, cross section of the outer cortex of the stipe showing radial rows of cells. Section 8 microns thick. ×55.—Fig. 7. C. triplicata, cross section of the inner cortex of the stipe where radial arrangement of cells is indistinguishable. At P appears an end wall in which the pits are scattered indiscriminately. At the bottom is the edge of the medullary region. Compare with figure 48 of plate IV. Section 8 microns thick. ×125.—Fig. 8. Alaria tenuifolia, cross section of the inner cortex of the stipe in which radial arrangement of cells has become negligible. Pits in the end wall may be seen at P in their characteristic circular arrangement. Compare with figures 5 and 12; the former would fit below figure 8, the latter above. Also compare with figure 45 of plate IV. Section 10 microns thick. ×55.—Fig. 9. Laminaria saccharina, cross section of the cortex of the stipe. The cells are in radial rows, and filaments from the medulla at the bottom extend in the intercellular spaces of the inner cortex. Some of the trumpeted filaments run otherwise than axially and may be seen in the medulla. Compare with figures 10 and 11 and with figures 44, 47, and 50 of plate IV. Section 7 microns thick. ×55.—Fig. 10. L. saccharina, longitudinal section of the outer cortex of the stipe showing the epidermal meristem and pits (P) in tangential and end walls. Compare with figure 9 and with figures 44, 47, and 50 of plate IV. Section 5 microns thick. ×125.—Fig. 11. L. saccharina, longitudinal section of the medulla of the stipe showing abundance of trumpeted filaments (T) and other filaments running in all directions. Inner cortex is at upper side of the figure. Compare with figure 9. Section 5 microns thick. ×55.



a meristematic nature. This has been called an epidermal meristem (fig. 1, 4, 10), but it must be understood that this meristem is a region of several lavers of cells and not a single epidermal layer. The meristem commonly gives rise to radial rows of cells which may or may not appear radial in longitudinal section (fig. 10) but which when viewed in cross section (fig. 1, 6, 12) nearly always have cells, at least those of the outer cortical region, in radial rows. Epidermal meristem in all plants studied is composed of between five and ten lavers of cells, the outermost always being covered with a mucilage like coating. Diatoms are very frequently found imbedded in the coating on almost any part of the plant. The cells of the meristem are much smaller than those of the cortex and are isodiametric or somewhat shorter radially (fig. 1, 4, 6, 10, 12), Plastids are found in abundance in the surface layers and in some instances almost completely occupy the entire meristematic region. Activity of the meristem in all cases gives rise to a radially arranged cortex whose cells become successively larger in closer proximity to the medulla. The typical example may be cited in figures 5, 8, and 12, which are from Alaria.

Pits are present in the walls of all cells throughout the plant, but it is in the cortex (fig. 2, 7, 8, 42) that they become the most conspicuous. The epidermal meristem is not excepted in having pits, though they are often comparatively small and few in number. Because of the abundance of plastids, the pits are often masked unless the sections are exceedingly thin. Further detailed description of pits follows in the description of the cortex.

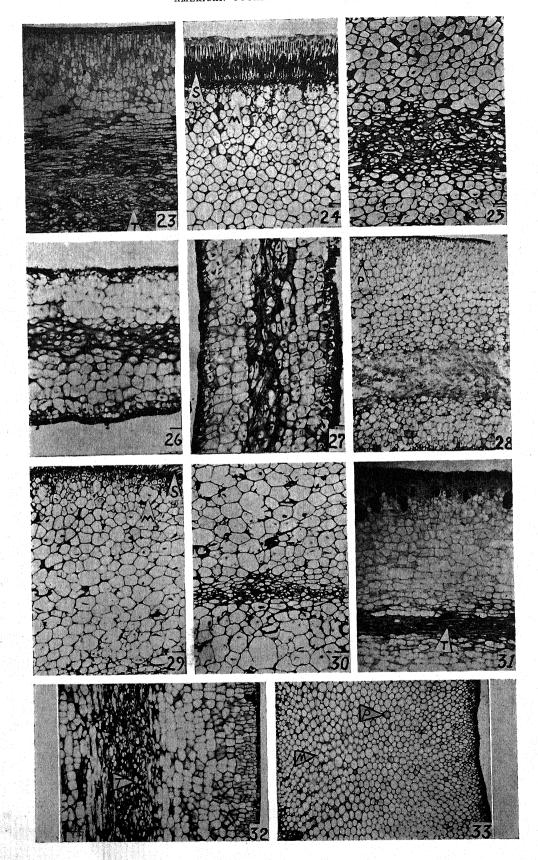
The cortical region (fig. 8) is to be understood as that region from the epidermal meristem inward, to a medulla (fig. 3, 5, 11) whose cells are usually of a smaller diameter and more elongate and where many branched filaments are frequently abundant. The cells of the cortex are approximately isodiametric and become only slightly longer as they near the medulla. The shape of the medulla may be elliptic in cross section as in Costaria, Alaria, Pterygophora, and Laminaria, or it may be circular as in Nereocystis and Cymathere. The shape of the medulla in cross section near a flattened transition region is nearly always elliptic, flattened in the plane of the flattening of the blade.

A distinction is often made between the inner and outer cortex, in which case the former refers to those cortical cells surrounding the medullary region, the cells of which may or may not be in radial rows. Sykes (1908) refers to those inner cortical cells in which radial arrangement is not perceptible as primary tissue and to those radial rows of cells added by the epidermal meristem as secondary tissue and termed outer cortex. This is apparently not infallible, because in some forms the radial arrangement goes into the medulla; especially is this so in Pterygophora, and in those forms with an elliptic medulla, the cortical cells adjacent to the ends of the ellipse are still in radial rows. However, since this paper does not take into consideration the development of tissues, no further discussion will be given here as to the feasibility of using the terms primary and secondary tissues. Outer cortex throughout this paper refers to those cells of the cortex from just within the epidermal meristem to a point halfway to the medulla.

The terms epidermal meristem, cortex, and medulla are the same as those used by previous workers.

All cells of the stipe have pits in their transverse walls, as is the case throughout the plant, and it is perhaps here that they are more commonly known

PLATE II.—Fig. 12-23.—Fig. 12. Alaria tenuifolia, cross section of the outer cortex of the stipe showing radial row arrangement. Compare with figures 5 and 8 of plate I. Section 10 microns thick. ×55.—Fig. 13. Nereocystis Luetkeana, cross section of the inner cortex and a portion of the medulla of the stipe in which appear a pitted end wall (P) of the cortex and a sieve plate (S) in one of the many sieve tubes of the medulla. The space between the cells of the medulla is filled with mucilaginous material. Compare with figures 36 and 39 of plate IV. Section 8 microns thick. ×55.—Fig. 14. N. Luetkeana, longitudinal section of the inner cortex and the outer medulla of the stipe showing typical sieve tubes at S and fragments of small interwoven filaments. Compare with figure 13 and figures 36 and 39 of plate IV. Section 5 microns thick. ×55.—Fig. 15. Pterygophora californica, cross section of medulla of the stipe showing typical arrangement of filaments, nearly all of which are trumpeted. Compare with figure 16. Note the somewhat gradual transition from cortex to medulla. Section 10 microns thick. ×46.—Fig. 16. P. californica, longitudinal section of juncture of medulla and cortex of the stipe showing the medulla composed almost entirely of trumpet filaments. Note the transition from cortex to medulla. Compare with figure 15. Section 15-20 microns thick. ×55.—Fig. 17. Costaria costata, longitudinal section of tip of a haptere showing the epidermal meristem and elongated cells of the medulla. Section 5 microns thick. ×55.—Fig. 18. Nereocystis Luetkeana, cross section of a haptere showing epidermal meristem and typical cell arrangement. Compare with figure 17. Stained with fast green and a red filter used. Section 10 microns thick. ×230.—Fig. 19. Laminaria saccharina, longitudinal section of medulla (at bottom) and cortex (at top) of the haptere showing elongated cells of the former and epidermal meristem of the latter. Section 10 microns thick. ×55.-Fig. 20. L. saccharina, longitudinal section of cortex in the crotch (C indicates direction of branching) of a dichotomously branching haptere showing the epidermal meristem and elongated cortical cells. Compare with figures 19 and 21. Section 10 microns thick. ×55.—Fig. 21. L. saccharina, longitudinal section of branching medulla in the haptere just behind location of figure 20 showing the nature of the branching medulla at M. The dichotomous haptere branches are off to the upper and lower right sides of the picture. Compare with figures 19 and 20. Section 10 microns thick. ×55.—Fig. 22. L. saccharina, cross section of the medulla of the transition region of the stipe and blade showing typical arrangement of filaments. Filaments may be seen extending into intercellular spaces of the inner cortex as at H. Compare with figure 23 of plate III. Section 5 microns thick.



than elsewhere. With the exception of Cymathere, the pits in the end wall are arranged in a circle around the edge (fig. 42, 45, 47). In Cymathere triplicata (fig. 7, 48) the pits are arranged in an indiscriminate fashion over the end wall. No explanation has been found for the difference or similarity of the pattern of pit distribution. The pits in the tangential and radial walls of the cells seem to follow no definite pattern of arrangement, but in many instances the circular arrangement is frequently found. Pits are relatively abundant in the tangential walls (fig. 10, 51) but are almost lacking in radial walls. This perhaps indicates that radial conduction is of nearly as much importance as longitudinal conduction and that tangential conduction is not carried on to any great extent through pits in the radial walls. Pits may vary in size from 1 micron to nearly the diameter of the whole cell, but the majority of them are 3 to 5 microns in diameter. Smallest pits are found in Alaria. The pits have a thin membrane across them in all cases. The membrane stains only slightly in safranin, and no stain was found in which excellent preparations for detailed study could be made. Bits of protoplasm are easily seen clinging to the membrane (fig. 42), and in some cases where there is a crack in the wall the stained pit membrane can be detected readily. Sykes (1908) reports sieve fields in the pits of Laminaria and Macrocustis, but the author is unable to see holes in the pit membrane even under magnification of 1800 diameters, nor does there seem to be any indication that such is the case in any forms studied, even including Laminaria saccharina on which Sykes reported. Since the pits themselves are even so small in diameter as to be only one micron at times, any holes through the membrane must be extremely small.

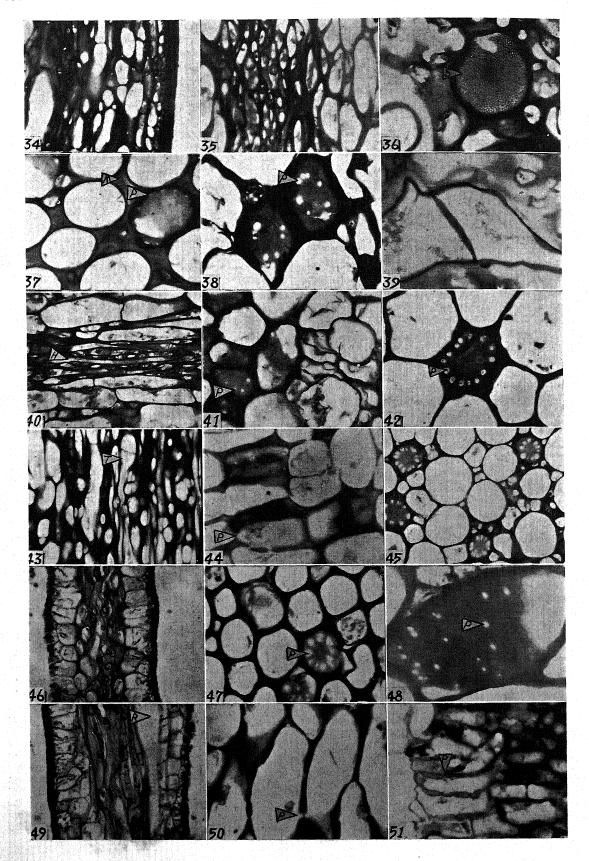
The number of pits in an end wall is not definite (fig. 45), and in all the plants observed may vary from a few to as many as 20.

Other than the increase in length and often lack of definite radial arrangement of cells, the only difference between the structure of the outer and inner cortex appears to be in the less compactness of cells nearer the medulla. When cells are not compact, all the forms studied have small diameter filamentous cells present in their mucilaginous intercellular spaces (fig. 7, 9, 45). This condition is common near the medulla. In some cases (Alaria and Costaria) the cells of the medulla are not elongate, and of course in these the inner cortical cells remain short and nearly isodiametrical.

The cortex of Pterygophora is unique among the forms studied in having concentric rings of tissue which have been called annual growth rings (Frye, 1918). The cells in the rings which would correspond to the summer wood of woody Spermatophytes are slightly smaller and appear to be much more densely filled with protoplasm than the other cortical cells. Pits present throughout the cells of the stipe are of the type found in the other forms (fig. 51). Other than the presence of the seasonal growth rings in the cortex there is no essential difference between the histology of the stipe of Pterygophora and the other plants discussed herein.

The medulla is different in the various forms studied. The medulla of *Nereocystis* is hollow in the center for most of its length through the stipe, and the cellular portion is rather narrow in comparison to the thickness of the other tissues. The other plants have a solid medulla but with the filamentous cells of varying compactness. Within the inner cortex of *Nereocystis* are axial filaments composed of cells of the same diameter as the inner cortical cells (fig.

PLATE III .- Fig. 23-33 .- Fig. 23. Laminaria saccharina, longitudinal section of medulla and cortex of the transition region of the stipe and blade showing numerous trumpet filaments of the medulla (T) and the gradation into short cells of the cortex. The epidermal meristem appears dark. Compare with figure 22. Section 4 microns thick. ×55.-Fig. 24. Cymathere triplicata, cross section of the outer cortex of the transition region of the stipe and blade showing outer region bearing a layer of sporangia (S). M represents mucilage ducts just beneath epidermis. Note that the isodiametric cells of the cortex are not in distinctly radial rows. Section 8 microns thick. ×55.—Fig. 25. C. triplicata, cross section of the medulla of the transition region of stipe and blade. Note cells of inner cortex and trumpet filaments in the medulla. Section 8 microns thick. ×55.—Fig. 26. Costaria costata, cross section of non-costate portion of the blade showing middle layer of trumpet filaments. The outer layers of cells are of the same thickness and are isodiametric. No mucilage ducts are present. Compare with figure 27. Section 8 microns thick. ×125.—Fig. 27. C. costata, longitudinal section of non-costate portion of blade. See below figure 26 for description. Section 8 microns thick. X125.—Fig. 28. C. costata, cross section of a costate portion of the blade showing middle layer of slightly trumpeted filaments, the outer layer on the ridged side of the blade (top) about twice as thick as that of the fold side, and a pitted end wall appearing at P. Section 8 microns thick. ×55.—Fig. 29. Cymathere triplicata, cross section of outer layer of the blade. Sporangia are present at S, and a mucilage duct may be seen at M. The cells are isodiametric, and outer layers on both sides of the medulla (compare with figure 30) are of the same thickness. Section 8 microns thick. ×55.—Fig. 30. C. triplicata, cross section of middle layer of the blade in which are present slightly trumpeted filaments. Compare with figure 40 of plate IV. On either side of the middle layer are isodiametric cells of the two outer layers. Section 8 microns thick. ×230.—Fig. 31. Pterygophora californica, longitudinal section of the middle and an outer layer of the blade. Trumpet filaments (T) are present throughout the medulla. Section 10 microns thick. ×55.—Fig. 32. Laminaria saccharina, longitudinal section of part of the blade showing middle layer composed of trumpet hyphae (T) and an outer layer of nearly isodiametric cells. Compare with figure 43 of plate IV. Section 10 microns thick. ×55.—Fig. 33. Alaria tenuifolia, cross section from the medulla to the surface of the midrib of the blade showing pits in the outer layer, especially at P and large intercellular spaces near middle layer filled with mucilage M. Extremely small cells to the left are those of the middle layer. Compare with figures 34 and 37 of plate IV. Section 7.5 microns thick. ×55.



14) but three to five times longer. These filaments are not compact, and among them are interspersed small, branching, non-trumpeted filaments which run in all directions. Intercellular material is mucilaginous and absorbs the stain readily. The cross walls of the large medullary filaments are true sieve plates (fig. 13, 36), and hence the term sieve tube has been used in discussing them (Oltmanns, 1904; Oliver, 1887; Rosenthal, 1890; Sykes, 1908; Will, 1884; and Wille, 1897). Sieve tubes in the medulla are only slightly trumpeted (fig. 14), and the protoplasm within is nucleate. Long filamentous cells with prominent fluted ends such as are present in Pterygophora (fig. 16) are not frequently seen in the stipe of Nereocystis. Branches are occasionally found in the sieve tubes, and when present merely run parallel with the main sieve tube. No connections were seen between the sieve tubes and the small muchbranched non-trumpeted filaments among them. The many branched filaments of the medulla are connected with cells of the inner cortex, and their transverse walls have pits in a circular pattern similar to the cells in the cortex.

The medulla of *Costaria* (fig. 3) is made up of nearly isodiametric cells of various sizes, no trumpeted filaments of any sort being present. Pits in the typical circular pattern are present in all the end

walls throughout the medulla just as in every other part of the stipe. Mucilaginous material is not abundant, the stipe is fairly solid, and intercellular space is minimized. The small cells intermingling with the large ones are not filamentous as in *Nereocystis*, and it is the presence of these small cells which forms a demarcation between the inner cortex and medulla (fig. 3). The inner cortical cells are all nearly the same size (fig. 2, 3). Nothing even remotely resembling a sieve tube or sieve plate is present in the stipe of *Costaria*.

The stipe of Alaria has a medulla (fig. 5) quite similar to that of Costaria except that the cells are more elongate and intertwining. There is also a trumpeting of the cells, though not so pronounced as in Pterygophora (fig. 16). All the cells of the medulla have their end walls with the typical circular arrangement of pits just as in the rest of the plant, and there is nothing present which might be called a sieve plate.

A cross section of the stipe of Laminaria presents a view of the medulla that very closely resembles that of Alaria (fig. 5). In longitudinal view the medulla of Laminaria is much more abundant with elongated filaments which are very prominently dilated at their cross walls (fig. 11). At the outer portion of the solid medulla are found trumpeted fila-

PLATE IV.—Fig. 34-51.—Fig. 34. Alaria tenuifolia, cross section of a portion of the blade near the midrib showing the middle layer and one of the outer layers. The middle layer is very thin and is composed of a few trumpeted filaments. Section 7.5 microns thick. ×192.—Fig. 35. Pterygophora californica, cross section of the middle layer of the blade showing the trumpeted filaments in cross section. Compare with figure 31 of plate III. Section 8 microns thick. ×400.—Fig. 36. Nereocystis Luetkeana, cross section of medulla of stipe to show a sieve plate. The white area in the sieve plate is a portion of the plate that was cut away in sectioning. Compare with figure 39 and with figures 13 and 14 of plate II. Section 8 microns thick. ×440.—Fig. 37. Alaria tenuifolia, cross section from the midrib of the blade. Cells from vicinity of M in figure 33 of plate III to show thickness of cell walls, the intercellular space filled with mucilage (M), and pits (P) in the end wall. The large white area in the end wall is where part of that wall was cut away in sectioning; the pits are fairly small. Section 7.5 microns thick. ×400.—Fig. 38. Pterygophora californica, cross section of outer layer of the blade showing the typical circular arrangement of pits (P) in the end walls. Section 8 microns thick. ×400.—Fig. 39. Nereocystis Luetkeana, longitudinal section from the medulla of the stipe showing a sieve tube in which the sieve plate, sectioned at 3 microns, is turned obliquely, presenting a view of both its thickness and surface. Compare with figure 36 and with figure 14 of plate II. Section 3 microns thick. ×440.—Fig. 40. Cymathere triplicata, longitudinal section of the middle layer of the blade to show trumpeted filaments at H. Note the thinness of the middle layer and compare with figure 30 of plate III. Section 10 microns thick. ×192.—Fig. 41. Laminaria saccharina, cross section of outer layer of cells in the blade showing pits (P) in their end walls. The large white space in the end wall of the indicated cell is a part that was cut away in sectioning. Section 8 microns thick. ×400.—Fig. 42. Costaria costata, cross section of cortex of stipe showing typical circular arrangement of pits in the end wall. At P may be seen bits of protoplasm clinging to the membrane across the pit. Compare with figure 2 of plate I. Section 8 microns thick. ×400.—Fig. 43. Laminaria saccharina, longitudinal section of middle layer of blade showing trumpeted filaments (T). Compare with figure 32 of plate III. Section 10 microns thick. ×192.—Fig. 44. L. saccharina, longitudinal section of the outer layer of the blade sectioned I micron thick to show the membrane across pits (P) in cell walls. Compare with figures 41, 47, and 50. ×400.—Fig. 45. Alaria tenuifolia, cross section of cortex of the stipe to show typical circular arrangement of pits in the end walls. Compare with area of P in figure 8 of plate I. Section 10 microns thick. ×192.—Fig. 46. Nereocystis Luetkeana, cross section of the blade showing the middle layer and the thin outer layers. Compare with figure 49. Section 8 microns thick. X192.—Fig. 47. Laminaria saccharina, cross section of cortex of stipe to show pits in their typical circular arrangement in the end walls. Compare with figure 50. Section 7 microns thick. ×95.—Fig. 48. Cymathere triplicata, cross section from the cortex of the stipe showing the end of one of the cells in which the pits (P) are scattered indiscriminately over the wall. The white area on the right side of the picture is where part of the wall was cut away in sectioning. Compare with figure 7 of plate I. Section 8 microns thick. ×400.—Fig. 49. Nereocystis Luetkeana, longitudinal section of the blade showing the middle layer of slightly trumpeted filaments and the thin outer layers. R represents a space where the outer layer separated somewhat from the middle layer in slide preparation. Section 8 microns thick. ×192.—Fig. 50. Laminaria saccharina, longitudinal section of cortex of the stipe showing pits (P) in tangential walls. Compare with figure 47. Section 5 microns thick. ×400.—Fig. 51. Pterygophora californica, longitudinal section of cortex of stipe showing pits (P) in tangential walls. Compare with pits in end walls of the blade in figure 38. Section 15-20 microns thick. ×192.

ments running a much straighter axial course than in the inner medulla and which gradate into the elongate non-trumpeted cells of the inner cortex. Although the trumpeted filaments in the outer medulla do branch at their cross walls, it is in the inner medulla that the filaments branch more profusely. These branches in some cases extend all the way across the medulla and into the intercellular spaces of the inner cortex (fig. 9). All the filaments of the medulla have pits in their cross walls or else these pits have become a single membrane (a single pit) stretching across the whole cell. The latter condition appears to happen frequently, but it is in Laminaria that it is found to occur more than in any other plant examined. Note in figures 47 and 45 how the pits are so close together that a single thin membrane would not be a great step removed from the condition existing there. Sykes (1908) explained this as a step in the formation of sieve plates, but as far as the investigator has been able to discern there are no holes such as are present in the sieve plate of Nereocystis; he is content to think of the membranes as pit membranes. Other than this membrane, Laminaria does not show evidence of having sieve tubes, especially in the sense that Nereocystis and Macrocystis (Sykes, 1908; Will, 1884) have.

A glance at the medulla of Cymathere shows it to be terete and set off from the inner cortex rather abruptly (fig. 7) by the decrease in cell size. Filamentous cells are much intertwined in the medulla, and though their sizes vary considerably, none of them are conspicuously trumpeted. Pits in the end walls of the cells are scattered in an indiscriminate pattern just as throughout the other cells of the plant (fig. 7, 48). Nothing even remotely suggesting a sieve plate or sieve tube is to be seen in the stipe of

Pterygophora has many long, very prominently trumpeted filaments and many short, muchbranched, non-trumpeted ones forming the much entangled mass of medulla (fig. 15, 16). There is a gradual transition between the cells of the inner cortex and those of the medulla. This transition is marked by the diminishing length and flutedness of the inner cortical cells. The trumpeted filaments have cross walls with the typical circular arrangement of pits just as throughout the stipe. No semblance of sieve tubes is noted.

In the much-branched non-trumpeted filaments forming entanglements in the medulla of all forms studied the end walls are pitted, and the remaining cylindric walls have no pits except at branching cells. These pits are in the typical circular arrangement in all except Cymathere, which of course has the indiscriminate distribution of pits throughout.

THE HAPTERE.—All of the species examined except Cymathere, have a holdfast composed of branching hapteres. Cymathere has a discoid holdfast. The hapteres arise from the base of the stipe and are usually rather closely intertwined. The epidermal meristem is nearly always thinner than that of the stipe and is almost imperceptible in Costaria and Laminaria (fig. 20). In Pterygophora the epidermal meristem is nearly as thick as in the stipe and in the older hapteres gives rise to concentric growth rings just as in the stipe. The haptere of Nereocystis (fig. 18) shows a fairly well defined epidermal meristem, usually much thicker than in the other species except Pterygophora.

The tip of a haptere has no special meristematic region other than the epidermal meristem which is not different than elsewhere (fig. 17). In the crotch of a branching haptere (fig. 20) there is also the same epidermal meristem. Nothing resembling a single apical cell is present in the haptere. The epidermal meristem, just as in the stipe, usually gives rise to radial rows of cortical cells. Pterygophora hapteres may have concentric growth rings present just as in the stipe, depending of course upon their age. Cells of the cortex in every case are long nearer the center of the haptere and are also usually larger

in diameter (figs. 17, 18, 19, 20).

There is no medulla of intertwining filaments resembling that of the stipe present in any of the hapteres. In longitudinal section there is no structural difference between the cortex and the very center of the haptere, other than the slightly longer cells in the center. In cross section the cortex always has radial rows of cells, the rows going all the way to the center in Nereocystis and very near the center in Pterygophora. When radial arrangement is not present, the center of the haptere resembles the inner cortex of the stipe in structure. In the center at a branch, as in Laminaria (fig. 21) the cells are seen to be running in two directions from the main axis without any difference in appearance of the cells in-

Pits in the end walls of all of the cells in the hapteres are in the typical circular arrangement and are scattered indiscriminately over the other walls. Pits are more numerous on the tangential than on the radial walls.

The base of the stipe has a medulla which is almost without semblance to that of the main part of the stipe. In this region the transition into hapteres is gradual, and in stipes with a medulla of elongate filamentous cells, the transition cells into the hapteres are merely elongate and closely resemble inner cortical cells. Where a haptere arises fairly high on the stipe, as is often the case, should there be a well defined medulla, there is no branching of that medulla into the haptere. The haptere arises at the outer surface of the cortex, and the cortical cells protrude into it. In the transition of stipe into hapteres in Nereocystis the sieve tubes gradate into merely elongate cells, and the medulla is not different from the cortex in structure. Nothing resembling sieve plates or sieve tubes is present in the hapteres of any of the plants examined.

In Cymathere, the representative with the discoid holdfast, the base of the stipe spreads out and is composed of isodiametrical cells all of nearly the same size. Differentiation into medulla and cortex is hardly distinguishable, but the epidermal meristem is still evident. The pits are present in all the walls and are not arranged in a circle as in the other algae examined but are scattered indiscriminately over the walls, as throughout the whole plant (fig. 48).

The transition region.—The tissues of the transition region between the stipe and blade are described in the same terms as used for the stipe—that is, epidermal meristem, outer cortex, inner cortex, and medulla. The meristematic transition region adds cells to the blade and stipe. Because of the many differences of the transition regions and blades of the species examined these will be treated separately rather than together as was done for the stipe and hapteres.

A cross section of the transition region of Laminaria is broadly elliptic in shape and flattened in the plane of flattening of the blade. The epidermal meristem and cortex of the transition region are hardly different from the stipe proper. No mucilage ducts are present here or in the stipe or hapteres. Other than the differences in shape of the tissues, comparisons of figures 9 and 10 of the stipe with figures 22 and 23 of the transition region show no important structural changes. Trumpet filaments are abundantly present throughout the medulla (fig. 23). Pits in the transition region are no different from those described in the stipe and hapteres, being abundant on the transverse and tangential walls but sparsely scattered over the radial walls. Filaments run radially into the cortex from the medulla (fig. 22) as in the stipe, but somewhat more prominently.

A broad thick transition region is characteristic of Cymathere, which also has a thick coriaceous blade. Mucilage ducts, not present in the stipe, make their appearance in the outer cortex of the transition region (fig. 24), and sporangia may or may not be present at the surface. The epidermal meristem does not give rise to distinct radial rows of cells (fig. 24) as is the case in the stipe (fig. 6). Cells throughout the cortex are nearly isodiametric (fig. 24), becoming only slightly elongate as they near the medulla. The medulla is relatively thin and very flatly elliptic in cross section. Its elements are made up of much entangled filaments many of which are trumpeted (fig. 25). In this plant the figures shown resemble the blade more closely than the stipe. Pits are still in the scattered rather than circular arrangement and differ in no manner from those of the stipe and hapteres. The pits on the transverse and tangential walls are more numerous than on the radial walls.

The transition region of Costaria is not essentially different in structure from that described for the stipe. Comparisons show that the medulla is relatively larger and less compact in the transition region and that the cells are less variable in size, somewhat elongate, and slightly trumpeted. The cortex is not very thick, and its radial arrangement is still discernible, indicating that an epidermal meristem is still functioning. Pits in the transition region are not different from those of the stipe and hapteres.

Alaria has a transition region in which the epidermal meristem is evident and which forms an outer cortex of cells in radial rows. No mucilage ducts are present, nor are they found elsewhere in the plant. A comparison of the transition region with the stipe yields few differences. The stipe is terete with an elliptic medulla, while the transition is elliptic in its entirety. The medulla of the transition region is less compact than that of the stipe. While the medulla of the stipe is made up of large and small cells, most of which are isodiametric, the medulla of the transition region is composed almost entirely of elongate trumpeted filaments. Many of these filaments extend into the intercellular spaces of the inner cortex as in Laminaria (fig. 9) and ultimately connect with cortical cells. Perhaps this abundance of trumpeted filaments in the transition region is partially accounted for by the fact that Alaria bears sporophylls here, as does Pterygophora.

Pterygophora has a broad flattened transition region bearing sporophylls and in cross section is similar to Alaria. The epidermal meristem forms radially arranged cortical cells, and evidence of production of concentric growth rings similar to those of the stipe may be found in the cortex. No mucilage ducts or cells are found, nor were they present in the stipe or hapteres. The structure of the medulla is not different in the transition region from that in the stipe, and figure 25 might well represent a section from the former rather than the latter. In the medulla, the trumpet filaments are abundant and the ends of the cells are prominently fluted.

The transition region of Nereocystis in the pneumatocyst of the stipe, and the bases of the leaf blades show mucilage ducts abundant in the outer cortex. Such mucilage ducts are not present in the stipe and hapteres. The outer cortex cells are in radial rows while those of the inner cortex are not definitely so. Typical pitted cells are present throughout the cortex as in the stipe, and the medulla is composed of sieve tubes with true sieve plates. Small, many-branched non-trumpeted filaments are also present in the medulla, just as in the stipe, but more compact in the former. No essential difference in structure is found between the stipe and the transition region.

The blade.—A section through a blade of all species examined shows three layers of tissues—a middle layer, corresponding to the medulla of the stipe, and a layer on either side of the middle layer corresponding to the cortex of the stipe (fig. 26, 27). The outermost cells appear to be nothing more than an epidermis, not resembling a meristem such as is found in the stipe. In the discussion of sections of the blade in this paper, the tissues will be referred to as middle layer and outer layers rather than as medulla and cortex.

The blade of Costaria has costae extending from the transition region to the tip. These costae are ridges on one side of the blade and folds on the other. On each side of the blade there is alternately a ridge, then a fold, etc. A cross section through a costa shows an outer layer twice as thick on the ridge side of the middle layer as on the fold side (fig. 28). By comparing a cross section (fig. 28) and a longitudinal section of the costa, the cells in the outer layers are seen to be almost isodiametrical, being only slightly elongate near the middle layer. The middle layer is made up of a loose mass of much-branched mostly non-trumpeted filaments; some of the filaments running axially are definitely trumpeted and frequently branched. Mucilaginous material occupies the intercellular spaces throughout the blade, being fairly abundant in the middle layer. There are no mucilage ducts present in the blade, nor any place else in the plant. The small non-trumpeted filaments frequently connect the two outer layers almost directly across the middle laver. The transverse walls of the middle and outer layers all have the characteristic circle of pits (fig. 28) as elsewhere in the plant. Pits are much more abundant in the transverse walls than in the other walls, being sparsely and indiscriminately scattered in the latter and not in the typical circular arrangement.

The inter-costal areas of the blade of Costaria resemble the costal portions just described except that both of the outer layers are of the same thickness (fig. 26, 27) and the cells of these outer layers are not elongated to any extent. The middle layer is made up of both trumpeted and non-trumpeted filaments, the latter being the more numerous. This layer in the inter-costal area of the blade is somewhat thinner and more compact than that of the costal area

Cymathere has a blade that is usually rather thick and coriaceous especially near its base. The blade has mucilage ducts (fig. 29) present in the outer layers which connect with those of the transition region. It will be remembered that the stipe has no mucilage ducts. The middle layer of cells is comparatively thin (fig. 30, 40) and is composed of much elongated trumpet filaments (fig. 40). In the outer layers the cells gradate (fig. 40) into the trumpet filaments of the middle laver. Pits in the transverse walls throughout the blade are abundant and of the type peculiar to Cymathere—that is, they are scattered and not arranged in a circle. A small number of scattered pits is present in the other walls. In figure 29 sporangia (S) are present at the surface of the blade, being just like those in the transition region (fig. 24).

A cross section of the blade of Pterygophora (fig. 35) is almost identical to that of Cymathere and needs no further explanation, but in longitudinal view (fig. 31) the blade shows the presence of mucilage cells rather than ducts in the outer layers. In Pterygophora (fig. 31) a rapid transition from the nearly isodiametric cells of the outer layer into the elongate trumpet filaments of the middle layer is seen. This is more marked than in Cymathere. Pits are abundant, comparatively large, and circularly arranged in the transverse walls (fig. 38) and sparsely and indiscriminately scattered in the other walls.

The blade of Laminaria contains mucilage ducts in the outer layer of isodiametric cells, the latter becoming longer as they near the middle layer (fig. 32). The middle layer is composed of much-branched filaments running in all directions, and many of those running longitudinally are trumpeted (fig. 32, T). Pits in the transverse walls are abundant, arranged in a circle, and are of the type found throughout the plant (fig. 41, 44).

In Alaria a percurrent midrib extending through the median axis of the blade is several times thicker than the expanded part of the blade. A cross section of an outer layer of the midrib (fig. 33) shows the cells much smaller toward the outside and much larger nearer the medulla. The intercellular spaces are fairly large and filled with mucilaginous material. Examination of longitudinal sections of the midrib proves that the cells of the outer layer are not isodiametric except at the epidermal layer, while the remaining cells are elongate to as much as five times their diameter near the middle laver. The transition is gradual until the middle layer is reached, where there are then present many muchbranched, trumpet filaments of small diameter. The expanded portion of the blade is very similar to that of Costaria (fig. 26, 34). The middle layer is comparatively wide, mucilaginous, and filled with trumpeted filaments, while the cells in the outer layers are somewhat elongate. Pits are much more abundant in the transverse walls than in the remaining walls and are in the typical circular arrangement (fig. 33, 37). Figures 33 and 37 show the thickness of the cell walls and mucilaginous material filling intercellular

Nereocystis has blades that are comparatively thin and in which the outer layers are only 2 to 5 cells thick (fig. 46, 49). The middle layer is also thin and is composed of branching filaments made up of slightly elongate, mostly non-trumpeted cells (fig. 49). Mucilaginous material is abundant in the middle layer. Cell walls with sieve plates (fig. 36) are not present in the blade as in the stipe. The typical pitted walls in which the pits are arranged in a circle are present, both in the middle and outer layers.

MICROCHEMICAL TESTS.—In testing for cellulose materials in the plants examined, it was found in every case that the cell walls were soluble (as is cellulose) in concentrated sulphuric acid and in concentrated chromic acid. The walls in Pterygophora were dissolved with difficulty. Cuprammonia acted upon the cells very slowly, and the tests yielded negative results for cellulose, the walls being insoluble. With iodine-potassium iodide following sulphuric acid, cellulose gives a blue color, but the test on cell walls yielded only a faint blue at its best and certainly not sufficient to indicate pure cellulose. The cell walls remained more yellowish-brown than blue. With chloro-zinc iodide solution the cell walls remained yellowish-brown which, were they cellulose, should become a violet color.

In only one species, Costaria costata, did the iodine-potassium iodide test yield a blue color on in-

tercellular material. The color was definitely blue black, indicating the probable presence of some type of hemicellulose (Tunmann, 1913). This material did not turn violet in chloro-zinc iodide, nor blue in iodine-potassium iodide when previously treated with sulphuric acid. It was not colored by corralin soda but was colored by safranin, aniline blue, and methylene blue.

Corralin soda gave evidence of varying amounts of callose in all species, depending largely upon the age of the cells. When callose is found, it is nearly always in either trumpet filaments or sieve tubes or both. Costaria gave only slight indication of callose formation even in the older plants. Tests with chlorozinc iodide gave a brick red color, also indicating callose (Tunmann, 1913). The same material stained a brown color in iodine-potassium iodide. Sieve plates of Nereocystis turned brownish in iodine-potassium iodide, brick red in chloro-zinc iodide, slightly bluish in iodine-potassium iodide following sulphuric acid, and in corralin soda gave a red color in varying quantities and shades.

Discussion.—The statement made by Humphrey (1887) that the lamina or blade of Agarum Turneri is both anatomically and morphologically a terminal expansion of the stipe also holds true for the species discussed herein. This may be even more emphasized for these species since trumpeted filaments are present in the blade as well as in the stipe. They were not reported in the blade of Agarum. If it is assumed that the trumpet filaments are for conduction and, in the case of Costaria, the trumpet filaments are more abundant in the transition region and the blade than in the stipe, then it seems very logical that the blade is morphologically only a flattened stipe.

Comparative study of the hapteres, stipes, transition regions, and blades shows that they are all of the same type of cells, except perhaps the true sieve tubes of Nereocystis, but developing under different internal environmental conditions. As cortical cells are added by the epidermal meristem, the more central cells are not able to keep pace with them by cell division and are stretched into elongate cells. Stretching also gives rise to what Setchell (1891) terms pulled out pore canals to form connecting filaments. This is in agreement with Will (1884) and Rosenthal (1890). The cells of the medulla of Costaria stipes are not elongate because the stipe itself does not become very long even in the adult plant. This is in direct contrast to the case in Nereocystis. Similar pits are present throughout all cells of the plants studied, even including the epidermal cells. The pits in the transverse walls are in circular arrangement (fig. 47, 42, 45) and are scattered indiscriminately over the radial and tangential walls. There are two exceptions. Nereocystis has true sieve plates with many holes in them (fig. 36) present in the medulla, all the other cells being typical. The other exception to pits in circular arrangement is found in Cymathere in which they are scattered indiscriminately (fig. 48) over the transverse walls just as in the radial and tangential walls but in greater abundance. There appears to be no particular significance in the arrangement of pits except that they are more often similar than dissimilar in arrangement. Sieve tubes reported by Kibbe (1915) in Alaria fistulosa are not present in Alaria tenuifolia.

Again referring to the effect of internal environment upon cells, examination of sections of hapteres and of the outer cortex of the stipe shows that those tissues under compression and slower in elongating (fig. 21) do not have trumpeted cells or pulled out pore canals as is the case in the medulla of the stipe and the middle layer of the blade. Histologically then, the plants studied are true thalli, and there is no essential difference in the parts that make up the plants.

Pits are present in the cells of the epidermal meristem as well as in those of the medulla. There is a difference in size of pits, those in the younger outer cells being smaller and fewer than in the inner cells. The pits average 2 to 15 microns in diameter, being nearer the former figure in nearly all cases. Sykes (1908) suggests that in the formation of sieve tubes the pits converge to form a single membrane which then becomes perforated forming a sieve plate. In Laminaria saccharina, upon which Sykes worked, the author did find the pits larger than in the other species studied (fig. 47), and this was the only case of possibly convergent pits, but even here he found no holes through the membrane of the pits even under magnifications of 1800 diameters with sections cut only one micron thick.

It seems logical that only those filaments or cells having walls with holes in them should be called sieve tubes. Only such walls are found (in those plants studied) in *Nereocystis*, and here the term sieve tube does not appear to be a misnomer. These sieve tubes have nucleate cytoplasm, and there are no companion cells such as in Spermatophytes. The definition of a sieve tube does not have to exclude nuclei or include companion cells.

The walls of trumpeted filaments present in the stipe, transition region, and blade are pitted rather than perforated just as are cells throughout the plant, whether or not they are trumpeted. It seems no more logical, merely because they are elongate and trumpeted, to call these filaments true sieve tubes than to call the unelongated pitted cells of the cortex sieve tubes. They are of the same type but have developed under different internal environmental conditions. Pitted cells are common in various tissues of all types of Spermatophytes, but we do not call them sieve tubes. When Oliver (1887) worked on the obliteration of sieve tubes in Nereocystis and Macrocystis by callose, he found that not only did the true sieve tubes form callose but also the trumpeted filaments. He concluded that these sieve tubes and trumpeted filaments are not homologous. The method of callose formation is not known for certain, and there is no proof that the material must pass through holes in the cell wall. Perhaps it might be formed within the cell where it is found. Thus it seems that callose is not necessarily limited to true sieve tubes

Rosenthal (1890) suggests that lateral protuberances may arise on the walls of inner cortical cells, thus giving rise to small interwoven cells in the medulla. Such protuberances are often found in the species studied and are suggestive of what Rosenthal proposed, but the author did not trace the development of filaments penetrating the medulla from the cortex, or vice versa, whichever the case might be.

Setchell (1891) suggested that the pulling out of pore canals laterally, when cells separate in growth, may give rise to connecting filaments in Sacchoriza (Laminaria). This might be the case in any or all forms treated herein, and one can easily imagine how lateral connections can arise by division of the cell in the canal. Regardless of the method of development, the abundant, intertwining, non-trumpeted filaments of the medulla are different from true sieve tubes especially in that their cross walls have pits, not holes. This is apparently in contradiction to Sykes' (1908) findings but not to those of Oliver (1887). No pits are found in the medullary filaments other than on the cross walls, because the cells are usually connected only at their ends. The same is true of the trumpeted filaments which run a nearly straight axial course in most instances.

Thick walled inner cortical cells of the stipe as reported in Agarum Turneri (Humphrey, 1887), Macrocystis luxurians (Will, 1884), Laminaria saccharina, Laminaria digitata, and Alaria esculenta (Reinke, 1876), and Nereocystis Luetkeana (Oliver, 1887) are not present in the species the author has examined. Sykes (1908) states that the mucilage present in intercellular spaces, as in Alaria (fig. 37), is really much swollen wall material. Figure 37 shows the definite thickness of cell walls, be they primary or secondary, separated by mucilaginous material, called swollen wall material by Sykes (1908). The intercellular material commonly has small filaments running through it, especially in the inner cortex (fig. 9, 22, 37, 45), indicating that it is not penetrated with difficulty. Naturally when stretching takes place in the inner tissues as a result of growth toward the outside, more space is produced within. Since the cells may secrete mucilage, perhaps the intercellular material is secreted from the cells after the primary wall or middle lamella has been destroved. If this be the case, then the walls are not thick, but if the mucilaginous intercellular material is swollen walls, in many cases the cell walls are thick, especially where the cells are not compact. This intercellular material gives no tests definitely indicating that it is either cellulose or callose, but it is readily colored in many stains including methylene blue, aniline blue, and safranin. In the case of Costaria, the mucilaginous material stained dark blue with iodine-potassium iodide, perhaps indicating the presence of some type of hemicellulose (Tunmann, 1913). The wall proper, be it primary or secondary, gives various reactions to microchemical tests. Some indicate that it is of cellulose and some that there is no cellulose present. As the walls are unchanged in color with various iodine reagents, fungous cellulose or a similar cellulose derivative is suggested. Ruthenium red has been known to stain walls of Nereocystis (Karrer, 1916; Rigg, 1925), indicating that pectose is present, but the author made no tests with this stain. It appears that walls of the Laminariales are of a modified form of cellulose not commonly met with in higher plants. Karrer (1916) reports algin, a pectose-like material, present with cellulose in the cell wall. It is generally believed that the walls are of a pectose-like material accompanied by cellulose.

Callose deposits may appear in trumpeted filaments and sieve tubes in varying proportions, apparently depending largely upon the age of the cells involved. Results obtained from Laminariales studied by other workers and microchemical tests on the species included here seem to be conclusive that callose is a special carbohydrate material distinct from cellulose and pectose.

Oliver (1887) and Sykes (1908), in their work on obliteration of sieve tubes in Laminaria, Macrocystis, and Nereocystis, present excellent figures of callose formations. Such formations are also found in all the plants examined here, but in Costaria it is almost negligible. No completely obliterated trumpet filaments were found in Costaria.

Mucilage ducts may or may not be present in tissues of the stipe or blade; none are present in hapteres. They are of no particular importance in connection with this paper, and their presence is merely noted. However, attention may be called to the fact that special mucilage cells rather than ducts are present in the outer layers of the blades of *Pterygophora* (fig. 31).

### SUMMARY

The comparative histology of Costaria costata, Laminaria saccharina, Cymathere triplicata, Nereocystis Luetkeana, Alaria tenuifolia, and Pterygophora californica is presented upon the basis of their stipes, hapteres, transition regions, and blades, in that order. The stipe, haptere, transition region, and blade are all of essentially the same type of cells except developed under different environmental conditions. The plant as a whole is nothing more than a thallus. The term "trumpet filament" is proposed to replace the term "trumpet hypha." Trumpeted filaments are not true sieve tubes, since their end walls are made up of pits rather than of a perforated membrane or plate. True sieve tubes have perforated sieve plates, nucleated cytoplasm, and no companion cells. True sieve tubes are reported only for Nereocystis among those species here examined. Pitted cells are present throughout the entire plant in all species considered. Pits are in a single circle in the transverse walls of all except Cymathere, in which case the pits are scattered indiscriminately over all the walls. In addition to pits in circular arrangement, Nereocystis has true sieve plates as end walls. Callose may be formed generally throughout the medullary cells but not in other cells of the plant. Cell wall material appears to be of modified cellulose of uncertain character.

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## NUCLEAR STRUCTURE AND BEHAVIOR IN SPECIES OF THE UREDINALES 1

### D. B. O. Savile

Anyone who studies the illustrations accompanying the many papers dealing with the cytology of the rust fungi must be impressed by two features common to all of them. The first of these is the remarkable variation, both in size and in appearance, of the rust nucleus in different organs and in different parts of the life-cycle. Very little comment is to be found concerning this variability. The second surprising feature is the remarkable dearth of convincing division figures elsewhere than in the basidium. In most stages no figures except a few telophases are to be found, and the interpretation of some of these is questionable.

It seemed to the writer that an elucidation of the nuclear structure and behavior throughout the lifecycle might greatly help our understanding of the general biology of the rusts and also clarify the imperfectly understood diploidization process. An attempt has accordingly been made to interpret nuclear behavior in the group as a whole, employing a number of species for study, rather than to elucidate what might prove to be the peculiarities of a single species.

A start was made on the investigation under the direction of Prof. J. G. Coulson, in the Department of Plant Pathology, Macdonald College, McGill University, during the winter of 1935-1936, at which time the writer held a Macdonald Alumni Scholarship. The bulk of the work was done at the Univer-

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sity of Michigan, under the direction of Prof. E. B. Mains, during the winters of 1937-1938 and 1938-1939; during this period the writer held the Emma J. Cole Fellowship in Botany. The writer takes this opportunity to express his sincere gratitude for the support that has made the investigation possible.

The writer is deeply grateful to Prof. H. J. Brodie, of the University of Manitoba and formerly of Macdonald College, and to Prof. B. M. Davis and Prof. W. R. Taylor, of the University of Michigan, for their valuable interest and advice throughout the work. Thanks are also due to the many others who have given advice at various times.

HISTORICAL SURVEY. — Cytological investigation of the rusts extends back to the work of Schmitz (1880), who discovered conjugate nuclei in the mycelium and urediniospores of Coleosporium Campanulae. The first extensive and important work in this field, however, did not appear until Sappin-Trouffy (1896) described and illustrated nuclear division in a number of species. This paper is of great importance because it indicated the fusion of the paired nuclei and the subsequent reduction division in the teliospore.

The progress in this field of investigation may be divided into three periods. In the first, started by Sappin-Trouffy, the nuclear fusion in the teliospore was stressed, and the origin of the dikaryon was overlooked. Sappin-Trouffy's work was followed by similar studies by Juel (1898) and Holden and

Harper (1903).

The second period started when Blackman (1904) described the initiation of the dikaryon at the base of the aecial primordium of Phragmidium violaceum by the migration of the nucleus of one cell into an adjacent one through a narrow pore. Shortly after this announcement Christman (1905) reported a slightly different method of diploidization in Phragmidium speciosum; namely, the fusion of two adjacent cells into a single large binucleate cell that gave rise to the aeciospore chain. These findings stimulated great interest in rust cytology, and during the next twenty years the aecial stages of some forty species were examined by Olive (1908), Kursanov (1910, 1922), Fromme (1914), and Lindfors (1924), to name the most important investigators. Nuclear migration was reported in a few species, equal-cell fusion in the majority, and both methods were seen in a number of species distributed through several genera. There was considerable disagreement concerning the relative significance of the two methods. Christman, Fromme, Kursanov (1910), and Olive all considered the few cases of nuclear migration seen by them to be pathological; but Blackman (1904), Blackman and Fraser (1906), Welsford (1915), and Kursanov (1922) all described nuclear migration through a small pore unaccompanied by equal-cell fusion. Opinion also differed during this period concerning the nature of the sexual process and its bearing on the phylogeny of the Uredinales. All the theories advanced were founded upon the assumption that the pycniospores were function-

The discovery by Craigie (1927a, 1927b) that the pycniospores of Puccinia Helianthi and P. graminis are indeed functional ushered in the third period of rust cytology and immediately diverted the attention of uredinologists into new channels. He showed that aecia were produced when the pycnial nectar of different pustules was mixed and spores thereby transferred; but that when this was not done and insects, which serve to transfer the spores in nature, were excluded, the pustules remained sterile. Papers by Craigie (1928, 1933), Hanna (1929), Allen (1930, 1931, 1932, 1933a, 1933c, 1934a, 1934b), Brown (1932, 1935), and Lamb (1935) clearly indicate that we may expect most of those rusts the pycnia of which are well developed to prove heterothallic. Ashworth's (1931) study of Puccinia Malvacearum gives weight to the belief that those forms with pycnia rudimentary or absent are homothallic.

Since the function of the pycniospores became known, various attempts have been made to demonstrate the mechanism whereby the pycniospore nuclei are transferred to the cells that they are to diploidize in the aecial primordium. Hanna (1929) suggested that the pycniospores of Puccinia graminis send germ-tubes down through the ostiole of the pycnium to which they have been transferred and form an independent mycelium the cells of which fuse with those of the aecial primordium. Allen (1934a) maintained the same to be true for Melampsora Lini.

Craigie (1933) demonstrated the fusion of pycniospores of *Puccinia Helianthi* with flexuous hyphae that emerge from the ostioles of the pycnia. These hyphae are distinguished from the stiff, tapering paraphyses by being blunt, flexuous, and very variable in length and abundance. Recently Buller (1938) has described the same process for *Puccinia graminis*.

Andrus (1931) saw what he termed trichogyne tips emerging from the stomata of bean leaves infected by *Uromyces appendiculatus* but presented no conclusive evidence that the pycniospores fused with them or that they led directly to the aecial primordium. Rice (1933) reported similar hyphae in the stomata of *Oxalis* leaves infected by *Puccinia Sorghi* and considered them to be trichogynes.

Lamb (1935), working with Puccinia Phragmitis, reported the fusion of pycniospores with ostiolar hyphae but could not distinguish the two distinct types described by Craigie. His illustrations, however, do show that some of the hyphae were fairly blunt at the tips, and it is possible that the flexuous hyphae were not well developed. He considered the passage of pycniospore nuclei from the ostiolar hyphae to the primordia, through the existing mycelium, to be the only method of diploidization by the pycniospores; but he noted that the coalescence of pustules of opposite strains also brought about the production of aeciospores. The latter method was demonstrated by Brown (1935) for P. Helianthi after he had already (1932) shown that diploidization could be effected in this way by a diploid pus-

Allen, working with Puccinia triticina (1931) and P. coronata (1932), described hyphae that push out not only through the stomata but between the epidermal cells close to the pycnia. She believed that the pycniospores fused with these hyphae and diploidized the small clusters of cells immediately beneath them. From these clusters the sporophyte was thought to spread to the aecial primordium both by the growth of these diploid hyphae and by the division and migration of nuclei through the existing mycelium.

Lamb's paper provides such an exhaustive discussion of the problem of sexuality in the rust fungi that to deal fully with the subject here could serve no useful purpose. He regarded the evidence for the presence of female sex organs to be very unconvincing and concluded that it was safest to avoid the use of the terms "male" and "female" and to regard the pycniospores as having a syngamic rather than a sexual function; that is to say, they are to be considered comparable to the oidia of the Hymenomycetes, the function of which was demonstrated by Brodie (1931, 1936). His viewpoint is thus in contrast with that of Andrus and Rice, each of whom regarded the rusts as having both true spermogonia and trichogynes. Andrus also spoke of the basal cells in the aecium as egg cells.

MATERIALS AND METHODS.—The following rusts have been included in the study: Uromyces Fabae

(Pers.) de Bary, U. Lespedezae-procumbentis (Schw.) Curt., U. Hyperici (Spreng.) Curt., Puccinia Sorghi Schw., P. Malvacearum Bert., P. Hieracii (Schum.) Mart., Melampsora Bigelowii Thüm., and Tranzschelia fusca (Pers.) Diet.

Uromyces Fabae, U. Lespedezae-procumbentis, and Puccinia Sorghi supplied most of the material for the aecial stage: the first from field collections and the others from greenhouse cultures with controlled mixing of the pycnial nectar. The teliospore germination stages were studied mainly from Puccinia Malvacearum, Melampsora Bigelowii, and Uromyces Lespedezae-procumbentis.

A wide variety of fixing and staining methods was tried, but the bulk of the material was handled by a few methods found suitable for the purpose.

In the descriptions of the figures the staining and fixing methods are given, in abbreviated form, except where no cytological detail is recorded. The abbreviations used are those given below.

The most generally used fixing solution was formalin-acetic-alcohol (F. A. A.: formaldehyde [40 per cent], 7 cc.; glacial acetic acid, 7 cc.; 50 per cent alcohol, 100 cc.). Somewhat different proportions were occasionally used, but this proved most generally useful, particularly where prompt embedding was not possible.

Chrom-acetic solution (Chr. Ac.: chromic anhydride, 0.5 g.; glacial acetic acid, 3 cc.; water, 100 cc.) was found useful with Heidenhain's haematoxylin for demonstrating certain details.

Duggar's (1909) modification of Gilson's fluid (D. G.) was used extensively because it is very suitable for material stained by the Feulgen method.

Two other solutions were used to a lesser extent—namely, acetic-alcohol (A. A.: glacial acetic acid, 20 cc.; 95 per cent alcohol, 80 cc.) and formalin-acetic-dioxan (F. A. D.: formaldehyde [40 per cent], 7 cc.; glacial acetic acid, 7 cc.; 50 per cent dioxan, 100 cc.).

While the appearance of the fixed nuclear structures was essentially similar after all fixations, the staining reactions of the differently fixed material varied greatly with certain staining methods.

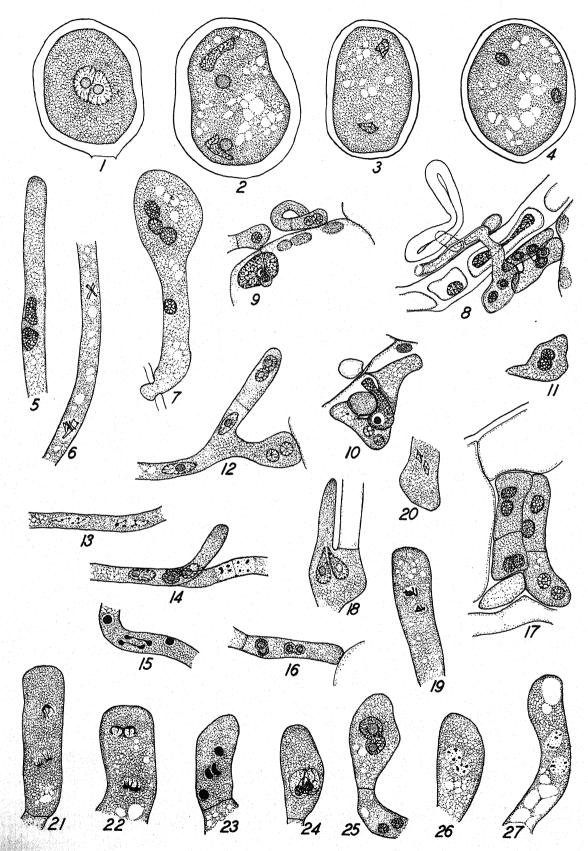
It was soon realized that the usual methods alone would not allow the complete solution of the problem, and an attempt was made to find methods that would supplement or replace them. While no one method was found to be universally applicable, two were discovered that, in conjunction with the generally used Heidenhain's haematoxylin schedule, cleared up most of the difficulties.

The first of these was based on the method described by Naylor (1926), but different dyes and a different buffer series were used. The buffer used was McIlvaine's disodium-phosphate citric-acid series, cited by Clark (1922). Sections of material fixed in F. A. A. were transferred from water to 0.5 per cent aqueous erythrosin for twenty minutes, dipped in water, and stained for twenty minutes in aqueous methylene blue. After a brief rinsing in water, they were transferred to a buffer solution di-

luted 1:50 from stock, at about pH 4.5, and left for about three hours. The buffer solution was changed twice during this period. Finally the slides were quickly rinsed in distilled water, dehydrated rapidly to avoid undue loss of dye, and mounted in xylol-balsam. Since the staining results are not markedly different over a range from about pH 4.0 to 5.0, elaborate precautions need not be taken in preparing the solutions. The stock solutions are most easily kept free from molds by covering them with a film of toluene. As might be expected, the reticulum of the host nucleus stains blue in contrast to red nucleoli and chloroplasts and red or purple cytoplasm. The rust nucleus, however, in most stages stains a bright red in strong contrast to the deep blue cytoplasm. As will be shown later, this surprising reaction seems to be due to the staining of ergastic material, perhaps mainly simple proteins, rather than the chromatin in the nuclei of the parasite. The writer has not had the opportunity to test the buffered methylene-blue eosinate method of Lillie and Pasternack (1936), which only recently came to his notice, but it would probably give results somewhat similar to this method. Several samples of each dye were used without apparent difference in the results. This method will be referred to as buffer staining, abbreviated in the description of figures to "buffer."

The second important method, new to rust investigations as far as the writer is aware, is the application of the Feulgen stain. The staining solution was prepared according to the formulae of both Margolena (1932) and de Tomasi (1936), but it was not possible to distinguish between the results. A single sample of basic fuchsin, unfortunately of unknown origin, was used throughout. The solutions kept perfectly. This method is not to be regarded as universally applicable, but it is extremely useful in studying the pycnial stage, in determining the precise distribution of chromatin within the nucleus, and in elucidating the nuclear divisions in the aecium. As the aeciospores, urediniospores, and teliospores approach maturity, some substance giving a red tinge to the cytoplasm is often present; the color is not identical with that of the chromatin, being a true red rather than purple, but it is close enough to it to hinder observation, particularly when the chromatin is finely dispersed. The method, therefore, is often disappointing for these stages, though good preparations are sometimes obtained. All fixing fluids except the chrom-acetic proved satisfactory with this method. It proved uniformly disappointing with all rusts and all host plants examined. Even with a long hydrolysis, which tends to loosen the sections from the slide, it was never possible to get more than a dull nuclear stain. The staining schedule used was that given by de Tomasi, fast green F. C. F. being used as a counterstain. This counterstain is to be understood in all cases, though the method will be referred to simply as Feulgen staining.

When the disposition of the chromatin is discussed, it is to be understood that by "chromatin" is meant the material, presumably nucleoprotein, that



reacts to the Feulgen stain. At the same time it is not to be inferred that nothing but nucleoprotein is involved in the movement; but in such minute nuclei it is in general only the chromatin that can be distinguished from the karyolymph.

Heidenhain's haematoxylin, usually with orange G as a counterstain, proved useful in the teliospore and basidium, particularly where it was desired to show spindle structure, and was used in other stages as a check on newer methods as well as to provide a comparison with the figures of earlier investigators. Its failure to render chromatin distinguishable from certain other materials, and the difficulty found in getting proper differentiation at certain stages owing to the accumulation of deeply stained ergastic material, are against its exclusive use: it should be used mainly when other methods fail.

Another point that should be more generally remembered is that the apparent size of minute structures stained by this method may be far greater than the true size. This is particularly true when chromic fixing fluids have been used. It is possible by this method to make the delicate spindle fibers distinctly visible, although in reality they are probably below the limit of vision. The method is similar to the mordanting and precipitation techniques used in demonstrating bacterial flagella. Other structures are similarly affected, and a stain heavy enough to demonstrate the spindle often causes the chromosomes to appear as a single clump rather than as individuals. No other haematoxylin technique having been used,

the method will be referred to simply as haematoxy-lin staining.

Other methods occasionally used have been the following: Safranin counterstained with fast green in clove oil; various modifications of Flemming's triple stain; aqueous methylene blue counterstained with erythrosin in clove oil; and Gram crystal violet counterstained with orange G. The Gram formula used was the rapid method of Dr. Guilford B. Reed, as given by Racicot, Savile, and Conners (1938). Its main use was in delimiting the inner nuclear sphere in certain stages.

The optimum thickness for paraffin sections in such a study as this was found to be 7  $\mu$ . Sections much thinner show few complete figures, and in sections appreciably thicker there is too much interference with the resolution of fine details, particularly if the more opaque dyes have been used.

The germination of aeciospores and urediniospores was studied mainly by germinating the spores upon thin agar films on slides. After appropriate intervals the preparations were fixed and were then handled like paraffin sections. Teliospore germination was studied in part by the use of sections and in part by scraping the germinating spores, or the basidia only in the case of *Melampsora*, into a drop of water upon a slide. After teasing the spores apart, a drop of fixing fluid was applied and allowed to dry, and the slide was then handled in the normal way. This apparently crude treatment sometimes yields remarkable results; even if half the basidia are lost, ample material remains if the germination was good.

Fig. 1-27. Unless otherwise stated, figures are reproduced at 1,920 diameters magnification. Abbreviations used are explained under "Materials and Methods."-Fig. 1. Nearly mature urediniospore of U. Fabae. Buffer. F. A. A.-Fig. 2. Discharge of endospheres from nuclei of U. Fabae urediniospore. Feulgen. A. A.—Fig. 3. Later stage than figure 2. Nuclei diminishing in size. Feulgen. A. A.—Fig. 4. Later stage than figure 3. Typical unexpanded nuclei. Feulgen. A. A.—Fig. 5. Unexpanded nuclei in germ-tube of U. Fabae urediniospore. Feulgen. F. A. A.—Fig. 6. Division of nuclei in germ-tube of U. Fabae urediniospore. Feulgen. F. A. A.-Fig. 7. Four unexpanded nuclei in germ-tube of U. Fabae urediniospore. Feulgen. F. A. A.-Fig. 8. Infection hypha of U. Fabae urediniospore. Collapsed spore seen on leaf surface. Several lobes formed on leaf surface before tube entered stoma. Three pairs of nuclei seen in the lobes in substomatal cavity, all partly expanded. Buffer. F. A. A. ×1480.—Fig. 9. Dikaryon U. Fabae hyphae forming haustoria in parenchyma cell. In each appressorium the partly expanded nuclei have reverted to the unexpanded form. In that at left cell wall has been penetrated, and one nucleus is passing into the young haustorium. Buffer. F. A. A. ×1480.—Fig. 10. Mature haustorium of U. Fabae coiling round and distorting nucleus of host cell. Haustorial nuclei still in unexpanded form. Buffer. F. A. A. ×1480.—Fig. 11. Mature haustorium of P. Hieracii. Nuclei unexpanded. Feulgen. A. A.—Fig. 12. Branched hypha of U. Fabae showing typical partly expanded nuclei above and unexpanded nuclei in lobe below where appressorium is forming prior to haustorium formation. Buffer. F. A. A.—Fig. 13. Anaphase in U. Fabae dikaryon mycelium. Feulgen. A. A.-Fig. 14. At right, late prophase in U. Fabae dikaryon hypha. At left, four partly expanded nuclei. Two in center, partly superimposed, are sliding past each other to restore the pairing of opposite strains; finally one pair would have moved out into new hyphal branch above. Buffer. F. A. A .-Fig. 15. Two nuclei sliding past each other in dikaryon hypha of P. Hieracii following simultaneous division. Nuclei still almost unexpanded. Haematoxylin, F. A. D.—Fig. 16. Stage shortly after division in hypha of U. Fabae. Crossing of nuclei has probably already occurred; otherwise nuclei would be farther apart. Buffer. F. A. A.-Fig. 17. Buffer cells at edge of U. Fabae uredinium. Nuclei slightly expanded, but endosphere does not show with this stain. Feulgen. A. A.—Fig. 18. Basal cell of U. Fabae forming lobe before dividing to cut off spore mother-cell. Pedicel of mature spore still attached above. Buffer. F. A. A .- Fig. 19. Prophase of division in urediniospore mother-cell of U. Fabae. Feulgen. A. A.—Fig. 20. Anaphase of division in basal cell of U. Fabae uredinium. Feulgen. A. A.— Fig. 21. Telophase in U. Fabae urediniospore mother-cell. Nuclei farther apart than length of spindle. Feulgen. A. A.— Fig. 22. Slightly later stage than figure 21; cutting out of endospheres from cytoplasm is in progress. Feulgen. A. A.-Fig. 23. Stage just after division in P. Antirrhini spore mother-cell. Two nuclei evidently distorted while in motion to restore pairing, spindles not having been side by side. Haematoxylin. Chr. Ac.—Fig. 24. Young U. Fabae urediniospore. Chromatin moving out into ectosphere. Feulgen. A. A.-Fig. 25. As figure 24 but spore nuclei almost fully expanded. Pedicel nuclei unexpanded. Feulgen. A. A.—Fig. 26. Prophase of division in U. Fabas urediniospore pedicel. Buffer, F. A. A.—Fig. 27. Anaphase of division in U. Fabae urediniospore pedicel. Buffer, F. A. A.

All examination of cytological detail was made with a Zeiss ×90 apochromatic objective of 1.3 N. A. and compensating eyepieces and with the condenser at about N. A.=1.2. The majority of the drawings were made with the  $\times 15$  eveniece and with the camera lucida adjusted to give a magnification of 2600 diameters on the drawing board; these are reproduced at a magnification of 1920 diameters. The few drawings reproduced at other magnifications have the scale indicated in the descriptions of the figures.

In the preparation of the figures it has sometimes been necessary to use slightly conventional methods, and these should be understood before the illustrations are studied. In the first place the limitations of monochrome rendering of details distinguished in the slide largely by color differences are quite serious. To make nuclear structures stand out from the surrounding cytoplasm, they have often been drawn somewhat more boldly than they appeared in the slide; but important dimensions, such as the diameters of the prophase chromosomes, have been reproduced as accurately as possible. In the second place, certain apparently amorphous structures, which cannot be reproduced naturally in a line drawing, have been indicated by a geometrical type of stippling to distinguish them from the reticulately stippled cytoplasm. Examples of this may be seen in figures 30, 31 and 34, and the method has also been used in a number of other drawings. Wall sculpturings have been left out in all drawings of spores since they are sometimes hard to distinguish without special methods, and they have no bearing on the present problem. For the sake of uniformity and to avoid further explanations, drawings have been oriented as though the host leaf were horizontal, and it has been assumed that all aecia opened on the lower surface and all other sori on the upper.

RESULTS.—The rust nucleus is commonly described and illustrated as having a relatively large nucleolus inside a reticulum that is very variable in both size and stainability. Sometimes the whole outer zone is represented as perfectly hyaline. At other times the entire nucleus is indicated as much smaller, being about the size of the so-called nucleolus, and it then contains either no visible nucleolus or only a very small granule the identity of which is hard to determine.

This variability puzzled the writer until a number of buffer-stained preparations were obtained in which a deeply-stained granule was visible in virtually every one of these inner spheres, or so-called nucleoli, of the larger, expanded type of nucleus (fig. 105). This granule could often be seen to be surrounded by an optically empty zone similar to the "heller Hof" commonly seen about the nucleolus in fixed nuclei of the higher plants. This at once suggested that the small granule, rather than the sphere in which it lay, was the nucleolus.

Attempts were made to secure adequate differentiation with haematoxylin to see how this inner sphere of the nucleus behaved during mitosis and the

subsequent nuclear reconstitution. Although by this means it was often possible to distinguish the granule within the inner sphere (fig. 103), it was seldom possible to discern the details of division owing to the presence, in dividing cells, of some substance with a great affinity for the haematoxylin. If destaining was carried far enough to clear all the cytoplasm. the nuclei were also almost completely destained. This was particularly true of the aecia, but it applied also to all other stages except the basidium, in which the nuclei are not of the expanded type.

It was not until the Feulgen method was used in an attempt to determine the precise distribution of the chromatin in the different types of nuclei that their real behavior began to be understood. Most of the features of the nuclear cycle have now been worked out, and in order to make them clear it is proposed to trace the entire life-cycle of the typical heterothallic, long-cycled rust, starting with the ure-

The morphology of the several fruiting structures has been described in detail for a number of genera by Moss (1926, 1929), Kursanov (1922), and Fromme (1914), among others, and will be taken up in the present paper only as it affects the problem at hand.

The uredinium.—The mature urediniospore has what will be termed the fully expanded type of nuclei (fig. 1)—that is to say, all the chromatin lies in the outer sphere, while the inner sphere is usually homogeneous in appearance and is a clear glassy green when the Feulgen stain is employed. It will soon be seen that these inner and outer spheres of the nucleus cannot be homologized with any parts of the nucleus of the higher plants and that they require distinctive names. It is proposed, therefore, to call them, respectively, the endosphere and ectosphere. These terms, while sufficiently descriptive to be useful, have the advantage of not implying any function that might later prove to be lacking.

Germination of the urediniospore is accompanied or preceded by a reversion of the nuclei to the unexpanded state—that is to say, they decrease in size and lack the endosphere, presenting instead the appearance of a small but typical nucleus. The change in form has been followed for Uromyces Fabae. The endosphere moves to one side until it lies against the ectosphere membrane. This membrane then breaks down locally and reforms behind the endosphere, which is accordingly left in a cupulate depression outside the nucleus. From here it drifts off into the cytoplasm (fig. 2) and eventually disintegrates. The greatly distorted nuclei now contract (fig. 3) and eventually round up into the typical unexpanded form (fig. 4). A minor disadvantage of the Feulgen method as here employed is that it is not possible to distinguish the true nucleolus of the unexpanded nucleus, everything but the chromatin being uniformly stained by the fast green. A recent paper by Semmens and Bhaduri (1939) describes a modification whereby the counterstain is removed from everything but the nucleolus; though satisfactory for chromosome studies in the higher plants, such a method would not meet the requirements of a problem of this kind. So far there has been no opportunity to perfect a differential stain for this purpose.

As germination proceeds, the nuclei move out into the germ-tube, either retaining the unexpanded form or becoming slightly expanded, with some of the chromatin outside what is then to be regarded as a new endosphere; but in either case the appearance with the Feulgen stain is unaltered, except that they may become somewhat distorted (fig. 5). If the host is not immediately penetrated the nuclei undergo simultaneous division in the germ-tube. The prophase of such a division is seen in figure 6. It will be noticed that the dividing nuclei are far apart, and this seems to be generally true. It is improbable that the nuclei ever divide side by side in the tube. Where two nuclei having a dumbbell shape are seen abreast in a narrow hypha or germ-tube, it is probable that they represent two daughter nuclei slipping past one another to restore the pairing of compatible strains after division; this device takes the place of the clamp-connection. After the division, unexpanded nuclei are again formed, as shown in figure 7; here, incidentally, a secondary spore is in process of formation, as often occurs when germination takes place under unfavorable conditions.

Figure 8 shows the invasion of a pea leaf by the germ-tube of a urediniospore of *Uromyces Fabae*. The empty, collapsed spore can be seen on the leaf surface. It will be seen that several lobes were thrown out by the germ-tube before one reached and entered the stoma. It is interesting to note that these lobes, as well as the first one formed in the substomatal cavity, all have distinctly thickened walls that stain like the spore wall, being green because of the combined effect of the methylene blue and the yellow wall pigment. This indicates that the infection process took a considerable time and that even in the substomatal cavity conditions were not entirely favorable.

Three pairs of nuclei are present in the hypha, all in the partly expanded state typical of nuclei in the vegetative mycelium. The endosphere is plainly seen with the buffer stain, but when the Feulgen stain is employed the even distribution of chromatin throughout the nucleus makes it indistinct. The exact condition of the hyphal nuclei varies considerably: there may be a fairly large ectosphere, containing most of the chromatin; or all the chromatin may be in the endosphere, and the ectosphere visible only as a small hyaline zone. Rarely they are completely unexpanded, but, in general, it may be said that they are partly expanded unless performing some special function.

In order to establish itself, the mycelium must send haustoria into the host cells. The first certain indication that a haustorium is to be formed by a hypha in contact with a host cell is the reversion of the nuclei to the unexpanded form (fig. 9, 12). This type of reversion is brought about by the retreat of such chromatin as is in the ectosphere into the endosphere. The ectosphere meanwhile contracts to meet the membrane of the slightly enlarging endosphere. The altered appearance is very striking with the buffer stain, but, of course, is not apparent in Feulgen preparations. An extremely slender penetration thread is formed, and, at its end, a lobe into which the nuclei pass. At the left of figure 9 the first of the two nuclei has passed almost completely into the lobe. The haustorium nuclei typically preserve their unexpanded form, though a suggestion of a small outer sphere is sometimes seen, as in figure 11.

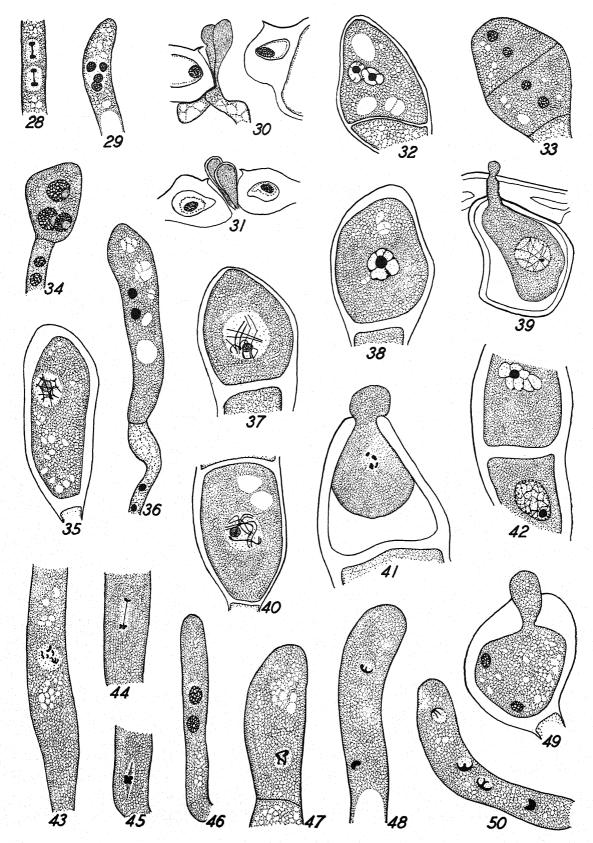
A mature haustorium of *Uromyces Fabae*, with unexpanded nuclei, is shown in figure 10, closely investing and distorting the host nucleus. That the haustoria of this species are attracted in some way toward the host nuclei seems to be quite certain. They may penetrate the cell at any point, but once they have entered it they very soon become directed toward the nucleus. One occasionally sees two or three widely separated haustoria converging upon a centrally located nucleus. The most logical explanation for this seems to be that certain elaborated food materials must be obtained from the host nucleus, from which they diffuse, rather than merely from the cytoplasm.

Clear and easily interpreted division figures in the mycelium are difficult to find. Those that have been found indicate that these divisions are similar to those to be described in more detail in connection with the basidium. At the right of figure 14, a pair of nuclei is seen in late prophase with the chromosome contraction completed. Of the four remaining nuclei the two in the center are partly superimposed and are sliding past each other to restore the pairing of compatible strains. The extra space provided by the new hyphal branch above has allowed this to take place without the usual amount of distortion. Eventually two of these nuclei would have moved out into this new branch. When the nuclei are still in the unexpanded state they seem to be very fluid and become greatly distorted in the process of rearrangement. This is shown in figure 15, which is the type of figure commonly regarded as a conjugate telophase. Such an interpretation looks upon the two stationary nuclei as persistent, discharged "nucleoli." The partly expanded nucleus does not discharge its endosphere, however; the endosphere merely increases slightly in size, and the division starts within it, and later its membrane breaks down.

When four nuclei are seen in a cell just after division, it does not seem possible to tell from the degree of their expansion whether the rearrangement has taken place. If, however, the nuclei are very closely associated in pairs, as in figure 16, it is probably safe to assume that it has already occurred, for sister nuclei are mutually repulsive and so would be separated by at least the length of the spindle.

Figure 13 shows a pair of hyphal nuclei in anaphase. The divisions in the two nuclei usually proceed at exactly the same pace, as in this example.

When a new uredinium is formed, a number of thin-walled but turgid buffer cells are produced first



at its center and later at its edges for the purpose of lifting the epidermis and allowing unhindered development of the spores. The nuclei of these cells stand out in sharp contrast to those of the spores (fig. 17), being only very slightly expanded.

Disregarding the general morphology of the uredinium, we turn next to the divisions leading to the production of the spores. These are two in number: that by which the basal cell cuts off the spore mothercell and that by which the mother-cell is separated into spore and pedicel. These divisions have never been followed accurately, only the telophase usually being depicted. They are considerably more difficult to distinguish than those in the aecium, which will later be described in detail, but it is thought that they are essentially similar. The nuclei of both the basal cell and the spore mother-cell become completely expanded and so must almost certainly divide in the manner typical for this form, with the prophase chromosomes lying outside the endosphere in the ectosphere cavity. The spore mother-cells are successively budded off from the basal cell, as shown in figure 18, a new one being formed laterally before the pedicel of the previous spore has disappeared.

Figure 19 shows the nuclei in prophase in a spore mother-cell; the chromosomes have contracted slightly, and the ectosphere membranes and the endospheres have disappeared. A basal cell in anaphase is shown in figure 20; the spindles appear slightly shorter than they actually are, because they are not in the exact plane of the slide. The telophase and nuclear reconstruction can be clearly followed and, together with similar stages at other parts of the life-cycle, supply further evidence that the endosphere is very different from the nucleolus as it is properly understood. The separating chromosome groups become arranged more or less in a square and take on the appearance of a flat plate from which delicate strands are often seen to trail (fig. 21). They move considerably farther apart than the length of the spindle, and then (fig. 22) curved processes of chromatin are thrown out from the chromatin plate to cut out a sphere from the cytoplasm. There are apparently about four of these processes; this suggests that they may actually be the chromosomes returning to the metabolic state. They soon anastomose to form a reticulate hollow sphere.

In these divisions there is usually plenty of room for the spindles to be formed side by side, and this position is commonly attained before telophase starts. Occasionally the orientation apparently fails to take place, and the nuclei are partly reorganized before pairing at the top and bottom of the cell. Figure 23 probably represents such a rearrangement; though, this being a haematoxylin preparation, it is not possible to be certain that the spherical bodies are not endospheres and the two bodies in the center imperfectly differentiated prophase figures. One argument against such an interpretation is that during the actual divisions it is usually impossible to get the cytoplasm as clear as in this figure and still keep the nuclei black.

After the second division, the two pairs of nuclei behave differently. Those in the pedicel remain unexpanded or virtually so, part of the chromatin passing into the central part of the nucleus and giving it an evenly reticlulate appearance (fig. 25). The chromatin of the spore nuclei may all remain for a short time at the periphery of the sphere cut out from the cytoplasm, or some of it may pass into its center; but before long a large ectosphere is delimited from the cytoplasm by the formation of vacuoles that gradually coalesce (fig. 24), the chromatin passes out toward the periphery of this, and the endosphere is soon left without any. The chromatin may nearly all pass to the outer membrane of the ectosphere or it may remain in a regular reticulum (fig. 25).

An interesting phenomenon rather frequently seen in *Uromyces Fabae* is the division of the pedicel nuclei. Four reformed nuclei have been found only once (fig. 29), and the telophases (fig. 28) are not common, but the earlier stages have been found

Fig. 28-50.—Fig. 28. Telophase of division in U. Fabae urediniospore pedicel. Buffer. F. A. A .-- Fig. 29. Four reconstituted nuclei in U. Fabae urediniospore pedicel. Buffer. F. A. A.—Fig. 30. Hyphal tips emerging from stoma close to U. Fabae uredinium, end pinched off in closing of stoma.—Fig. 31. Hyphae similar to those in figure 30, in lifted epidermis over U. Fabae uredinium.—Fig. 32. Partly expanded dikaryon nuclei in young teliospore of P. Malvacearum. Haematoxylin. Chr. Ac.--Fig. 33. Unexpanded nuclei in young teliospore of P. Hieracii. Haematoxylin. F. A. D.—Fig. 34. Young teliospore of T. fusca, showing early expansion of nuclei. Those of upper cell have already fused although septum between cells has not yet formed. Feulgen. F. A. A.-Fig. 35. Fusion nucleus in U. Fabae teliospore, showing leptonema threads. Feulgen. A. A.-Fig. 36. P. Malvacearum teliospore before final division. Nuclei unexpanded. Haematoxylin. F. A. D.-Fig. 37. Zygonema stage in P. Malvacearum teliospore. Haematoxylin. Chr. Ac.-Fig. 38. Partly expanded fusion nucleus in P. Malvacearum teliospore. Haematoxylin. Chr. Ac.-Fig. 39. Germinating M. Bigelowii teliospore in Salix leaf. Nucleus approaching leptonema. Haematoxylin. F. A. A.-Fig. 40. Zygonema in P. Malvacearum teliospore. Four pairs of threads seen. Haematoxylin Chr. Ac.-Fig. 41. Diakinesis in germinating P. Malvacearum teliospore. Chromosomes too closely paired for individuals to be discerned. Haematoxylin. F. A. A.-Fig. 42. P. Malvacearum teliospore, showing lower nucleus completely expanded while ectosphere of upper nucleus is still forming and little chromatin seems to have left endosphere. Haematoxylin. Chr. Ac.-Fig. 43. Diakinesis in P. Malvacearum basidium. Haematoxylin. Chr. Ac.—Fig. 44. Late anaphase of first division in P. Malvacearum basidium. Haematoxylin. Chr. Ac.—Fig. 45. Metaphase in first division in P. Malvacearum basidium. Haematoxylin. Chr. Ac.—Fig. 46. Unexpanded interphase nuclei in U. Lespedezae-procumbentis basidium. Feulgen. D. G.—Fig. 47. Prophase of second division in P. Malvacearum basidium. Haematoxylin. Chr. Ac.—Fig. 48. Late telophase of first division in P. Malvacearum basidium. Haematoxylin. F. A. D.-Fig. 49. Unexpanded interphase nuclei within germinating teliospore of U. Lespedezae-procumbentis. Feulgen. F. A. A.-Fig. 50. Late telophase of second division in M. Bigelowii basidium. Haematoxylin. F. A. A.

rather frequently in some collections. A late prophase is shown in figure 26, and an anaphase in figure 27. These figures stain well with the buffer method and are clearer than those usually obtained in the hyphae. This is partly explained by the small amount of ergastic material found in these cells and partly, perhaps, by the scanty cytoplasm. The reason for this seemingly functionless division is not certain. The only apparent clue to its origin that the writer has been able to find is in Fromme's (1912) investigation of *Melampsora Lini*. He showed that the urediniospore pedicels of this rust are commonly two- or three-celled. The divisions in *U. Fabae* may be a relic of this condition.

Associated with the uredinial stage in some of the material of *U. Fabae* were rather numerous hyphal tips emerging from the stomata. These seem to have been short-lived, since nearly all those seen had been pinched off by the closing stomata, as in figure 30. The contents are amorphous in appearance rather than granular or reticulate, and so were presumably not living at the time of fixation. These hyphae are very similar in appearance to those seen by Andrus (1931) associated with the pycnia of Uromyces appendiculatus, and by Rice (1933) associated with the pyenia of Puccinia Sorghi. Both these writers considered them to be trichogyne tips. Ashworth (1935a) showed that such hyphae occur close to the uredinia or telia of several rusts, including some that are apparently homothallic. These findings clearly indicate that such hyphae, though they may possibly serve as receptive hyphae, are not to be regarded as specific female structures. Actually they seem to be formed wherever the mycelium is massed under the epidermis and their emergence is probably largely a matter of chance, though it may be influenced by the external environment. A careful examination of the ruptured epidermis over the uredinia revealed many such tips broken off and lodged in the stomata (fig. 31).

Allen (1935) described what she considered to be conidia cut off from the apices of stomatal hyphae of *Puccinia Malvacearum* and suggested that they might take the place of the pycniospores in this rust. As Ashworth (1931) showed fairly convincingly, however, that this rust is homothallic, and so not in need of such spores, it seems probable that what Allen saw were hyphal tips cut off in the manner here described.

The telium and basidium.—The development of the telium in Puccinia and Uromyces, with which we are chiefly concerned, is essentially the same as that of the uredinium. In Puccinia there is one extra division in the formation of the two-celled spores, but this division presents nothing new. The nuclei eventually become expanded before this final division, but they may remain unexpanded for some time (fig. 36), while the cell is increasing in size. The next stage of importance comes immediately after this final division in the formation of the teliospore, when the nuclei become expanded and fuse. There seems to be great variation in the rate of these processes, in

relation both to each other and to the development of the spore wall. Figures 33 and 34 form a remarkable contrast. Figure 33 shows a young spore of Puccinia Hieracii; here the formation of the ectospheres has not yet started, although the septum between the cells is complete. The teliospore of Transchelia fusca is also two-celled. In the example shown in figure 34 not only have all the nuclei become fully expanded, but those of the upper cell have already fused, although no sign of a septum could be seen. Figures 34 and 42 provide a further contrast. The nuclei of the lower cell in figure 34 have become completely expanded before fusion. In figure 42, representing a teliospore of Puccinia Malvacearum, the two nuclei of the upper cell have fused, although expansion is not nearly complete.

The process of nuclear expansion is very clearly seen in the teliospore. As already mentioned it is initiated by the formation of a number of vacuoles around the unexpanded nucleus. The irregular shape seen in the upper cell of figure 42 is rather exceptional, but slightly lobed outer zones, such as are shown in figures 32 and 38, are commonly seen. The vacuoles gradually coalesce and the newly-formed ectosphere takes on a smooth periphery. At the same time, the chromatin gradually leaves the endosphere, passing along the protoplasmic strands in which it is supported. Finally the chromatin becomes disposed in a typical open reticulum, as in the lower cell in figure 42.

The delicacy of the chromatin strands makes difficult the exact identification of the early stages of the first meiotic division in the fusion nucleus, but some have been clearly recognized. During the expansion the endosphere decreases somewhat in size. It may continue to dwindle and to be almost completely disintegrated at the start of prophase (fig. 39); but as a rule it persists almost unchanged until the pairing of the elongated chromosomes is complete. The endosphere then disintegrates rather rapidly, as is shown both by its decreased size and by its decreased stainability.

The first indication of the start of prophase is the change of the chromatin from a reticulum to a number of strands that at first seem to have no wellordered arrangement and whose individuality is not clear (fig. 39). A slightly later stage is shown, for Uromyces Fabae, in figure 35. In this spore the threads are more distinct, but they seem to be distributed at random throughout the ectosphere. Their exact number cannot be ascertained, since it is often impossible to distinguish two adjacent threads from a single sharply bent one. The next recognizable stage is the zygonema, of which several good examples have been found in Puccinia Malvacearum. In figure 37 the number of chromosomes is in doubt, because the pairing is not complete and the threads are greatly contorted toward the basal side of the nucleus. In figure 40, however, there seems to be little doubt that the number of pairs is four, since all the ends lie free from one another. The exact length of the threads cannot be determined, since their convolutions are in both the vertical and horizontal planes, but they are surprisingly long. One pair seems to be about 8  $\mu$  in length, a second about 6  $\mu$ , and the others from 4 to 5  $\mu$ .

The chromosomes apparently contract very abruptly after pairing, for it has not been possible to find the next prophase stages with the exception of an occasional rather indistinct diakinesis. Actually the chance of discerning any doubleness of the threads in the missing stages with such unfavorable material is small. Figure 41 shows the nucleus about to pass into the basidium in what is evidently diakinesis; there are two small and two relatively large clumps of chromatin.

As far as could be judged from the material examined, the stage at which the nucleus leaves the teliospore varies somewhat with the species. In Melampsora Bigelowii it generally seems to migrate before the pairing of the chromosomes (fig. 39). In Puccinia Malvacearum it generally passes into the basidium between zygonema and diakinesis, whereas in Uromyces Lespendezae-procumbentis division may become far advanced in the spore and may even be completed within it (fig. 49). It is not certain that this is an inherent tendency of the species, since it may conceivably be influenced by the physiological state of the material at the time of germination.

The stage shown in figure 43 is not very easily identified, but since the individual chromosomes are distinct it is presumably somewhat later than that seen in figure 41, and immediately precedes the formation of the spindle. The metaphase itself is clearly seen in material heavily stained with haematoxylin (fig. 45), as is the succeeding anaphase, but the heavy stain required to bring out the spindle usually makes a chromosome count impossible by causing the chromosomes to appear clumped.

In late anaphase it often looks as though there were only two chromosomes going to each pole, and this effect in particular seems to account for the chromosome count of two given by Sappin-Trouffy for all species examined by him. Careful focussing reveals that each body is actually two superimposed chromosomes. This is shown in the late anaphase seen in figure 44. At the upper end of the spindle there seems to be merely a two-lobed chromatin mass; but the lower end is viewed somewhat obliquely, and it is seen that there are four bodies arranged approximately in a square, though even here only two of the group could be brought into focus at the same time.

Occasionally end views of anaphases have been seen in sections of germinating Puccinia Malvacearum sori, and these confirm the arrangement of the chromosome groups in a square. Such views do not lend themselves to illustration, but it is possible to draw the two groups separately, which shows that there are two very small chromosomes and two slightly larger ones in each group. The larger ones, allowing for the foreshortening effect that obtains when they do not lie quite parallel to the slide, have been calculated to be about 0.5–0.7  $\mu$  long, and the

shorter ones seem to be about 0.25  $\mu$  long. The diameter of each seems to be about 0.20–0.25  $\mu$ .

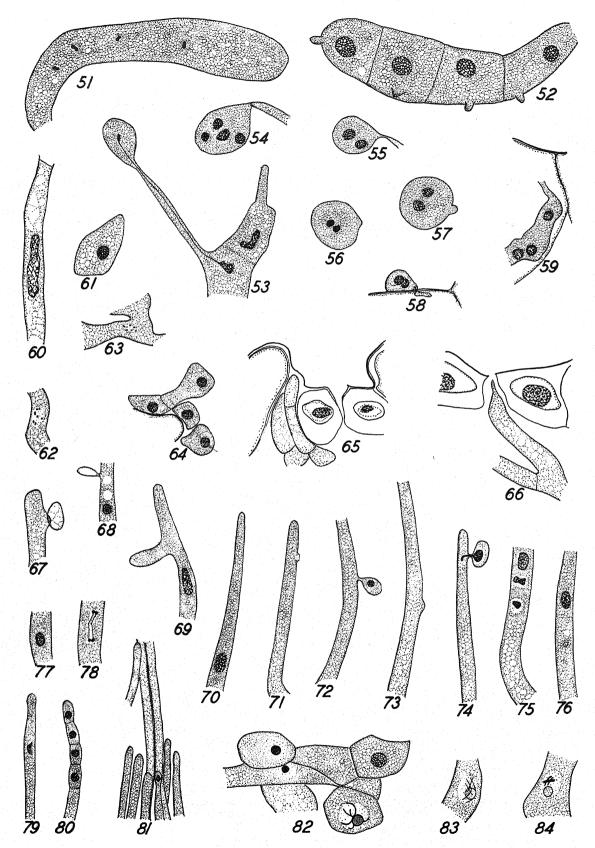
It is at first difficult to see how such long threads as those shown in figure 40 can contract in anaphase to the dimensions indicated. The explanation probably is that the apparent diameter of the prophase threads is much greater than the true diameter. Two factors may contribute to such an effect. In the first place, the diameter is increased by the adsorption or precipitation of dye and, perhaps, other material upon the chromosomes. Though both the long and the contracted chromosomes will be affected, the proportionate increase will be far greater for the thin thread. In the second place, the diameter of the stained prophase threads, though just above the limit of actual vision, is considerably below the limit of true resolution. With green light the resolving power of an objective with a numerical aperture of 1.3 is about 0.2  $\mu$  with a full condenser cone. Objects appreciably narrower than this are usually invisible if lightly colored, but if deeply stained they will be discernible and will appear considerably broader than they really are. If the prophase threads contract without change in volume, their calculated diameter when at their maximum length is about 0.04 μ. The contraction probably is accompanied by some loss in volume, but even so the true minimum diameter must be considerably less than 0.1  $\mu$ .

No well-defined centrosomes have been seen in any of the forms studied, though small dots are sometimes visible at the ends of the spindle in haematoxylin preparations. These may be centrosomes, but it seems possible that they are simply optical effects. The spindle fibers, even when heavily stained, are so far below the limit of resolution that they appear gray; but where they meet at the end of the spindle they may build up to a size approaching the limit of resolution and so appear black.

Allen (1933b), in her study of the germination of the teliospores of Puccinia Malvacearum, suggested that the haploid number was five. The chromosomes are so small that it is often impossible to be certain whether one is looking at one relatively large, or two adjacent, very small ones. The writer's observations, gleaned from both prophase and anaphase figures, indicate a haploid number of four for this species and for Uromyces Fabae, U. Hyperici, Puccinia Sorghi, and Melampsora Bigelowii. Observations on other species do not warrant an estimate.

The groups of chromosomes usually move far apart during telophase (fig. 48) and cut out new spheres from the cytoplasm in the manner already described. The nuclei become reticulate but remain unexpanded (fig. 46). To judge from the small number of metabolic nuclei seen at this stage, the interphase must be short.

The second division is typical of that of unexpanded nuclei in other parts of the life-cycle. In the prophase seen in figure 47, chromosome contraction had probably already commenced, since the threads are rather thick; but in this division, and in all the mitotic divisions, it seems that the prophase chromo-



somes attain only about half the length of those seen in the fusion nucleus.

The period at which the first septum is laid down in the basidium varies greatly, but it has never been seen to form until all traces of the spindle have disappeared. It may be formed at the time of the prophase of the second division (fig. 47), but it is commonly delayed until the telophase (fig. 51), or even nuclear reconstruction following this division (fig. 50). Nuclear reconstruction is similar to that following the first division, and the septa between the products of the second division form promptly, resulting in a four-celled basidium with unexpanded nuclei. For some reason these nuclei seldom stain strongly even with haematoxylin or the Feulgen stain, and occasionally they are indistinguishable. Not only is the ergastic material, on which the buffer stain is dependent, almost entirely absent, but the chromatin seems to be in a more delicate reticulum than usual, and the threads may be below the limit of vision.

The formation of the sterigmata starts as soon as the basidium becomes four-celled. Their length depends largely upon whether or not there is water upon the leaf surface. If the basidium is almost completely immersed, the lower sterigmata usually become very long, in order to raise the spores clear of the surface. The nucleus passes into the spore before the latter attains its full size, and becomes greatly constricted in passing through the apex of the sterigma. When the latter is longer than usual, this attenuation of the nucleus may be very remarkable (fig. 53).

Allen (1933b) has already shown for Puccinia Malvacearum that the basidiospore nucleus divides

before the germination of the spore. The present study has confirmed this and has shown the same to be true of Uromyces Lespedezae-procumbentis and Melampsora Bigelowii. Figure 56 shows the telophase of the division in M. Bigelowii, and figure 57 shows the two unexpanded nuclei. In U. Lespedezae-procumbentis the division commonly occurs before the spore is discharged (fig. 55), or even before it reaches its full size. On one occasion a quadrinucleate spore was found in this species (fig. 54). Since the basidium was not intact, it was not possible to determine whether this represented an extra division or whether two nuclei from the basidium had passed into the spore.

The monokaryon thallus.—The basidiospore germinates by forming a very short germ-tube and an appressorium and then enters the host by sending a slender process directly into an epidermal cell (fig. 58). One or more nuclear divisions normally occur after penetration; the intracellular hypha accordingly contains several unexpanded nuclei (fig. 59). Then septation occurs, and at least one of the cells pierces the inner wall with a slender thread, and its nucleus passes into the intercellular space to initiate the normal intercellular mycelium. Only then do the nuclei become partly expanded.

The nuclei of the monokaryon mycelium are essentially similar to those of the dikaryon. They range from the greatly extended form illustrated in figure 60, commonly found in old hyphal cells, to the unexpanded or scarcely expanded forms seen in the pseudoparenchyma cells bounding the aecial primordium (fig. 61) or the pycnium (fig. 64). Divisions are even harder to distinguish than in the di-

Fig. 51-84.—Fig. 51. Early telophase of second division in M. Bigelowii. Feulgen. F. A. A.—Fig. 52. Unexpanded nuclei after second division in M. Bigelowii basidium. Haematoxylin. F. A. A.—Fig. 53. Nucleus passing into U. Lespedezae-procumbentis basidiospore. Feulgen. F. A. A.-Fig. 54. Quadrinucleate U. Lespedezae-procumbentis basidicspore. Feulgen. F. A. A.-Fig. 55. Normal binucleate basidiospore of U. Lespedezae-procumbentis. Feulgen. F. A. A.—Fig. 56. Telophase of division in M. Bigelowii basidiospore. Feulgen. F. A. A.—Fig. 57. Normal unexpanded nuclei in M. Bigelowii basidiospore. Feulgen. F. A. A.-Fig. 58. P. Sorghi basidiospore penetrating epidermal cell of Oxalis leaf. Feulgen. F. A. A.—Fig. 59. Intracellular infection hypha of P. Sorghi; unexpanded nuclear form retained. Feulgen. F. A. A .- Fig. 60. Partly expanded nucleus in old hyphal cell of U. Lespedezae-procumbentis; endosphere not distinguishable with this stain. Feulgen. F. A. A.-Fig. 61. Unexpanded nucleus in pseudoparenchyma cell close to accium of U. Fabae. Feulgen. F. A. A.-Fig. 62. Anaphase figure near base of partly diploidized accium of U. Fabae. Buffer, F. A. A.-Fig. 63. Same as figure 62. Buffer, F. A. A.-Fig. 64. Unexpanded nuclei in pseudoparenchyma bordering pycnium of U. Lespedezae-procumbentis. Feulgen. F. A. A.-Fig. 65. Hyphae of P. Sorghi pushing between epidermal cells of Oxalis leaf. Feulgen. D. G.—Fig. 66. U. Fabae stomatal hypha with tip pinched off by closing of stoma.—Fig. 67. Empty U. Fabae pycniospore attached to papillate ostiolar hypha. Safranin. F. A. A.—Fig. 68. Partly collapsed P. Sorghi pycniospore attached to hypha. Feulgen. F. A. A.— Fig. 69. Branched blunt hypha emerging from P. Sorghi pycnium. Feulgen. D. G.-Fig. 70. Typical ostiolar paraphysis from P. Sorghi pycnium. Feulgen. F. A. A .- Fig. 71. Papillate ostiolar hypha of P. Sorghi; fixed four hours after mixing nectar.—Fig. 72. Pycniospore attached to P. Sorghi ostiolar hypha; fixed four hours after mixing nectar. Feulgen. D. G.—Fig. 73. Papillate ostiolar hypha of U. Lespedezae-procumbentis; fixed thirty hours after mixing nectar.—Fig. 74. U. Fabae pycniospore nucleus passing into slightly tapering hypha. Methylene-blue erythrosin. F. A. A .- Fig. 75. P. Sorghi ostiolar hypha with one relatively large hyphal nucleus and two small introduced nuclei; fixed four hours after mixing nectar. Feulgen. D. G.—Fig. 76. P. Sorghi ostiolar hypha with thin area on wall; fixed two hours after mixing nectar. Feulgen. F. A. A .- Fig. 77. Resting nucleus of U. Fabae pycniosporophore. Feulgen. F. A. A.—Fig. 78. Telophase of division in U. Fabae pycniosporophore. Feulgen. F. A. A.—Fig. 79. Prophase of division in U. Hyperici pycniosporophore Feulgen. F. A. A.-Fig. 80. Catenulate pycniospores of U. Lespedezae-procumbentis, showing unexpanded nuclei. Feulgen. F. A. A.—Fig. 81. Hyphae growing up into ostiole from base of U. Lespedezae-procumbentis pycnium. ×1330.—Fig. 82. Cells at base of P. Sorghi primordium prior to diploidization; at left, two unexpanded nuclei; at right above, partly expanded nucleus; below, nucleus with chromatin passing out to periphery of ectosphere. Feulgen. D. G.-Fig. 83. Prophase of division of expanded nucleus in P. Sorghi primordium. Feulgen. D. G.—Fig. 84. Slightly later stage than figure 83; same preparation.

karyon, where one of the two nuclei may be so oriented that the stage can be seen. Two anaphases, from close to the base of a young accium of *Uromyces Fabae* in which diploidization was just starting, are shown in figures 62 and 63. These will be further discussed in connection with the diploidization process.

The monokaryon haustoria are formed in essentially the same way as those of the dikaryon mycelium and, except for being uninucleate, are essentially similar in appearance. A single binucleate haustorium was found in a monokaryon pustule of Uromyces Fabae, but this is clearly to be regarded as an abnormality. The nuclei normally retain their unexpanded form, but a hyaline zone is sometimes seen around them, which probably indicates the initiation of the ectosphere. In very old haustoria they often take on an irregular form and may increase in size.

The pycnium and the introduction of pycniospore nuclei.—The pycnial stage is, of course, particularly interesting because of its importance in connection with the process of diploidization. Considerable attention was therefore devoted to it in an effort to find out more about the mechanism by which pycniospore nuclei of the compatible strain are brought to the aecial primordium. While this was not completely successful, some additional information was obtained that may help to lessen the existing confusion. A great deal of material of Puccinia Sorghi and Uromyces Fabae was examined, and smaller amounts of material of U. Lespedezae-procumbentis and U. Hyperici. The two latter, and U. Hyperici in particular, have smaller nuclei than the other species and so are less suitable.

Particular attention was devoted to the occurrence of emergent hyphae, whether of the stomatal, epidermal, or ostiolar type. The observations made add weight to the writer's opinion that the stomatal and epidermal hyphae are formed largely by chance. Stomatal hyphae were occasionally seen close to the pycnia, but always with their tips crushed by the stomatal movement (fig. 66). Hyphae pressing up between the epidermal cells were often seen (fig. 65), but never breaking through the cuticle. The remnants of both types can often be seen in the crushed epidermis bordering the ostiole of the pycnium, which strengthens the belief that they are the result of a massing of mycelium beneath the epidermis and are not specialized organs.

Several times whole pustules of Puccinia Sorghi and Uromyces Fabae, in which diploidization had just started, were carefully examined, and were found to be without any emergent hyphae, other than those from the ostioles. Although these epidermal and stomatal hyphae, if better developed, may serve as supplementary receptive hyphae, it seems clear that in these two species the ostiolar hyphae are considerably the more important.

Craigie (1933) and Buller (1938) distinguished two types of ostiolar hyphae: the stiff, tapering, marginal paraphyses, and thin-walled, flexuous hyphae with blunt ends, varying greatly in length and abundance and emerging more nearly from the middle of the ostiole. Only the latter were considered to be receptive. Their origin has not been clearly demonstrated, but it is logical to believe that they arise from lower on the wall of the pycnium than do the

marginal paraphyses.

Hunter (1936), in a morphological study of the pycnia of the Melampsoraceae, saw groups of such hyphae arising from the center of the pycnium in a number of species. She also pointed out that Colley (1918), to whom their function was naturally obscure, saw similar hyphae in Cronartium ribicola. In this family there are no paraphyses, and there is every reason to believe that Hunter's suggestion that these are receptive hyphae is correct. Moreover, free-hand sections revealed pycniospores clinging to them. No such large groups of hyphae seem to arise from the bases of the pycnia in the Pucciniaceae, but individual hyphae, or groups of two or three, may occasionally be found. Figure 81 shows parts of two old hyphae and the tip of a young one arising from the center of the base of a pycnium of Uromyces Lespedezae-procumbentis. The older hyphae could be followed to the ostiole in the adjacent section, but could not be traced to the end. If a hypha passes completely through the congested region within a single section it may be possible to trace its entire length, but as a rule only free-hand sections permit determination of the full extent of the apical part of a long emergent hypha. These, unfortunately, are virtually useless for following it through the ostiole. That the flexuous hyphae do not always arise from the base of the pycnium in the species studied is evident, however, for many pycnia, with hyphae that are definitely of the flexuous type, show no hyphae arising from lower than about halfway up the sides of the cavity. The lowest of such hyphae usually emerge approximately in the center of the ostiole.

Not all writers have been able to distinguish the two types of ostiolar hyphae, a fact that is partly explained by the rarity with which a complete hypha of any great length will occur intact in a paraffin section; but the problem is further complicated by the frequent lack of a sharp distinction between the two types. In all the species studied, the writer found that if all the hyphae in a central section were drawn, there was nearly always a complete gradation from the typical paraphysis (fig. 70) at the edge to the blunt and often branched hypha toward the center. It is not possible to divide them into the two clearly separate types shown by Buller (1938) in his sketch of the pycnia of *Puccinia graminis*.

The most satisfactory stain for this phase of a cytological study of the rusts is unquestionably Feulgen's. The pycniospores stain very poorly with the buffer stain and not much better with haematoxylin. The fact that the cytoplasm of the ostiolar hyphae does not stain well adds further to the difficulty. The apical parts are invariably light pink when the buffer stain is used. This reaction suggests that the cytoplasm contains some of the ergastic material

that is normally found in the nuclei but is absent from those of the pycniospores. They are also often slightly tinged with pink when the Feulgen stain is used and never take the counterstain as strongly as does the vegetative mycelium. There is evidently some substance present that gives an aldehyde reaction; but whether it is really a nucleoprotein or merely some ergastic substance, perhaps formed as a result of the altered environment of the hyphae, is not clear.

The nuclei of the pycniospores are spherical and unexpanded (fig. 72, 80), those of the emergent hyphae elliptical and partly expanded (fig. 69, 70). The nuclei of the pycniosporophores are sometimes expanded to some extent, but are very often compact and apparently unexpanded (fig. 77). This compactness is explained by the fact that they divide so frequently to produce new spores that they have little time to expand. Prophase figures with the chromosomes lying approximately parallel (fig. 79) can be found, but the metaphase and anaphase stages have not been clearly distinguished. Telophases stain very clearly and may be found in almost any section. For some reason the spindle, just before it disappears, is often sharply bent (fig. 78).

Hunter (1936) describes the pycniospores as being typically catenulate in the Melampsoraceae. A tendency toward this habit was occasionally observed in *Uromyces Lespedezae-procumbentis* (fig. 80), but generally, in the species studied, one spore is discharged before the next one starts to form.

Very few convincing examples of pycniospores attached to receptive hyphae were found. Spores were often seen in contact with the hyphae, but it was only occasionally that definite evidence of union could be found. Figure 67 shows an empty spore of Uromyces Fabae attached to a large papilla near the tip of a blunt hypha. Figure 74 shows another fusion in U. Fabae, in this case with a slightly tapering hypha. This preparation was not well-stained, but the nucleus appears to be starting to migrate into the hypha. The fusion papilla is in this case much smaller. Figure 68 shows an empty and partially collapsed spore of Puccinia Sorghi attached to a slightly tapering hypha. The small rounded nucleus is probably that of the spore, the hyphal nucleus being lower down and out of the section. Well-marked vacuoles, such as are seen in this figure, often accompany great activity in a cell, and these suggest that nuclear migration had only just occurred. Figure 72 shows another fused pycniospore of P. Sorghi, this time with the nucleus still in the spore.

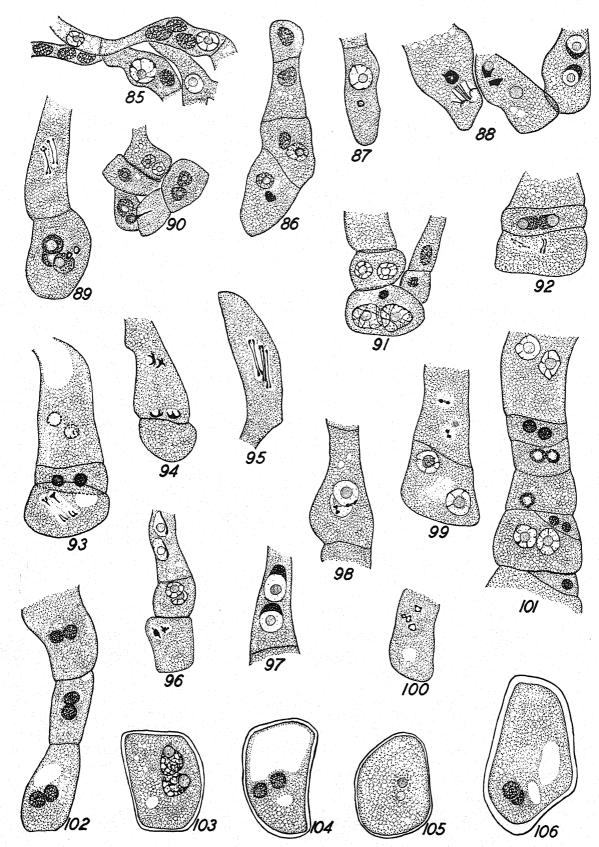
Branched hyphae (fig. 69) are not uncommonly seen in *P. Sorghi*. They seem to be essentially similar to those shown by Buller (1938) in his figure 3, but nothing approaching the length indicated by him has been seen in either paraffin or free-hand sections of any of the species examined. The length of these hyphae may be dependent upon the environmental conditions. Pycniospores were on several occasions seen in contact with the tips of these short side branches but were never so oriented that it could be

definitely decided whether they had actually fused to them or were merely in chance contact.

Two other evidences of the introduction of pycniospore nuclei through the ostiolar hyphae were found in material fixed a few hours after mixing the nectar. The first of these is the occurrence of small papillae on the more flexuous hyphae. These may appear rounded (fig. 73), or the top may be flattened and extremely delicate (fig. 71). What is evidently the same structure as this last may be seen in surface view. By focussing very carefully upon the upper or lower wall of a hypha, using not too strong a light, small circular bright areas may occasionally be seen. Figure 76 shows the typical size of such a spot: its actual appearance is beyond the scope of a line drawing. These thin areas and papillae evidently represent places at which pycniospores were attached at the time of fixation. The second additional evidence is the occasional presence of several nuclei in a hypha. They are likely to be somewhat distorted and abnormal in appearance, but react characteristically to the Feulgen stain. Two such nuclei are seen below the hyphal nucleus in figure 75. The abnormal shapes may be due to violent streaming associated with the passage of the introduced nuclei past the hyphal nucleus or may indicate the beginning of nuclear division. Since plurinucleate ostiolar hyphae are never found in pustules guarded from insects prior to mixing the nectar, it is evident that these are actually pycniospore nuclei.

The aecial primordium.—The development of the aecial primordium usually starts considerably before the first pycnia start discharging spores, but the degree of differentiation reached at the time of diploidization depends on the time that elapses between opening of the pycnia and mixing of the nectar. In nature the degree of differentiation is often very considerable, but if diploidization is effected very early, it may be slight. As a rule it is possible to distinguish the basal "fertile layer" and the outer "sterile layer," to use the terminology of the earlier writers. This outer tissue of very vacuolate cells has already served its purpose by thrusting aside and crushing the chlorenchyma cells, and it then gives up most of its nutritive material to the more active parts of the thallus and is destroyed when spore formation starts. It is possible for diploidization to start before this differentiation is evident, however, and this may partly account for the lack of agreement about the precise point of formation, in the young accium, of the first dikaryon cells.

At the base of the primordium typical hyphal cells are seen, usually with slightly expanded nuclei. As we progress toward the apex (downward in the drawings), we find a more or less pseudoparenchymatous structure in which it is often impossible to tell which are the consecutive cells of a hypha. The first of these may have almost unexpanded nuclei, but those a little nearer the epidermis usually have completely expanded nuclei at the time when diploidization starts. Finally, directly beneath the epidermis, lie the "sterile" buffer cells whose nuclei are



also nearly unexpanded. In a partly differentiated primordium the change of form of the nuclei can be studied. Figure 82 shows a group of cells from such a primordium of Puccinia Sorghi. The two dense nuclei at the left are completely unexpanded, though the hyaline zone surrounding each suggests that expansion might be about to start. At the right above is a partly expanded nucleus the chromatin of which is too evenly distributed for the boundary of the endosphere to be evident with the Feulgen stain. At the right below, nearest the leaf surface, is a nucleus in which the fully expanded form is being adopted. Here a large ectosphere has been delimited and the chromatin is passing out to its periphery along delicate protoplasmic strands. When the process is complete, most of the chromatin is usually very finely dispersed about the ectosphere membrane and may be only barely discernible with the Feulgen stain. This makes it very apparent that much more than chromatin is stained in the reticulum of the expanded nucleus when haematoxylin is used.

If diploidization is delayed beyond the time when these cells have become fully expanded, the primordium may continue to enlarge to some extent. This continued growth gives us an opportunity to observe the division of the expanded nucleus. The chromatin being all in the ectosphere, the division figure takes shape, not in a slightly enlarged endosphere, but in the ectosphere cavity. Figure 83 shows the chromosomes at about their maximum length, overlying the endosphere. Figure 84 shows a later prophase with the chromosomes partly contracted. The division of the expanded nucleus will be taken up in detail later, in connection with the formation of the aeciospores.

Diploidization.—If the nectar is mixed and compatible nuclei are introduced as soon as the pycnia

open, diploidization may start before the nuclei with which they are to pair are fully expanded. When, on the other hand, mixing of the nectar is delayed for some time, the majority of nuclei in the base of the primordium become fully expanded. The state of the primordium at the time of diploidization can thus be seen to have some bearing on the ease with which the thallus nuclei can be distinguished from those that have been introduced, for the migrating nuclei are unexpanded. This point is clearly seen if figures 85 and 86 are compared. Figure 85 shows a group of cells at the base of a primordium of Puccinia Sorghi, fixed twenty-four hours after the nectar was mixed. In each cell there is one fully expanded thallus nucleus, with a clear green endosphere supported by a few delicate strands in the large ectosphere with almost all the chromatin disposed on its periphery. Sharply contrasting with these expanded nuclei are a number of evenly reticulate, smaller nuclei that take the Feulgen stain very strongly. Three of these are to be seen in the cell at the left; four, partly superimposed, are in the cell at the upper right, and there is one in the center cell below.

Figure 86 shows a single hypha from a primordium of Uromyces Lespedezae-procumbentis in which diploidization had progressed to about the same extent. The whole development of the pycnial and aecial stages of this rust seems to be much slower than that of P. Sorghi. The retardation includes not only the time between the opening of the first pycnia and the full differentiation of the primordia, but apparently even the time taken by the introduced nuclei in reaching the primordium. In no instance was any diploidization observed within twenty-four hours of mixing the nectar, while in P. Sorghi the minimum is apparently slightly under ten hours.

Fig. 85-106.—Fig. 85. Aecial primordium of P. Sorghi twenty-four hours after mixing nectar; numerous unexpanded introduced nuclei present, contrasting strongly with the expanded thallus nuclei. Feulgen. F. A. A.-Fig. 86. Hypha in partly diploidized accium of U. Lespedezae-procumbentis. The terminal and subterminal cells each have one unexpanded and one almost fully expanded nucleus. Feulgen. F. A. A .- Fig. 87. Cell in P. Sorghi primordium twentyfour hours after mixing nectar; fully expanded thallus nucleus and small nucleus evidently in late prophase; the latter probably dividing to supply a nucleus for an adjacent cell. Feulgen. D. G.—Fig. 88. Conjugate divisions in partly diploidized aecium of U. Fabae; early prophase at right; later stage at center with ectosphere membrane broken down; at left, intermediate prophase stage, the four chromosomes in each nucleus still elongate and the two nuclei forming mirror images, while above there is a third nucleus in the cell. Feulgen. F. A. A .- Fig. 89. Same aecium as figure 88; terminal cell contains three expanded nuclei, while the fourth has apparently just divided to supply a nucleus to an adjacent cell. Feulgen. F. A. A.—Fig. 90. Nuclear migration at base of P. Sorghi aecium forty hours after mixing nectar. Feulgen. F. A. A.-Fig. 91. Chain of dikaryon cells in young U. Fabas accium with extra, unexpanded nucleus in one cell and monokaryon hypha adjacent. Feulgen. F. A. A.-Fig. 92. Late anaphase in U. Fabae aeciospore mother-cell, the spindles not yet parallel. Feulgen. F. A. A.—Fig. 93. U. Fabae aeciospore column, showing early telophase in spore mother-cell; the spindles are parallel. Feulgen. F. A. A.-Fig. 94. Late telophase in U. Fabae basal cell, showing formation of endospheres of new nuclei. Feulgen. F. A. A.—Fig. 95. Early telophase in trinucleate basal cell of  $\bar{U}$ . Fabae; this one originated deep in the primordium, but the majority are formed by equal-cell fusion of a dikaryon with a monokaryon cell. Feulgen. F. A. A.—Fig. 96. Early prophase in basal cell, and early anaphase in spore mother-cell of P. Sorghi. Feulgen. D. G .- Fig. 97. Early prophase in basal cell; same preparation as figure 96.—Fig. 98. Anaphase in P. Sorghi basal cell; endospheres and membranes of ectospheres visible at later stage than usual. Feulgen. D. G.-Fig. 99. Similar stage to last, but ectosphere membranes and one endosphere have disappeared; second endosphere is disintegrating. Same preparation as figure 98.—Fig. 100. End of anaphase in P. Sorghi basal cell; spindles not yet parallel. Feulgen. F. A. A.—Fig. 101. U. Fabae acciospore chain, showing variation in nuclear form. Feulgen. F. A. A .- Fig. 102. Chain of columnar cells in U. Fabae aecium. Feulgen. F. A. A.—Fig. 103. U. Fabae acciospore showing distinct granule, surrounded by "heller Hof," in each endosphere. Haematoxylin, with prolonged destaining. F. A. A.-Fig. 104. Peridial cell of U. Fabae. Haematoxylin. F. A. A.—Fig. 105. Peridial cell of U. Fabae with endospheres showing distinctly and chromatin indistinct. Granule visible in each endosphere. Buffer. F. A. A.-Fig. 106. Peridial cell of U. Fabae. Feulgen. F. A. A.

Although there is little doubt which are the thallus and which the introduced nuclei in the two binucleate cells in this figure, it would have been difficult to make such a distinction for the other cells had they contained more than one nucleus; and, indeed, one cannot be sure that the nucleus in the top cell, which runs out of the section, is actually the thallus nucleus.

There is no sign in figures 85 and 86 of any partial dissolution of the septa, nor was this seen in any material of this stage. The unexpanded nucleus is evidently adapted to passing through minute openings. The septal pores, though not usually visible in the mycelium, can be seen to be about  $0.15-0.20~\mu$  in diameter in the spores. Such an opening is probably ample for its passage, since that in the base of the haustorium is usually considerably narrower. In preparations steeped first in tannic acid and then in ferric chloride to give intensely stained walls, the septal pores in the older, vacuolate hyphae are seen to be filled by small plugs of undetermined composition. These are presumably destroyed or displaced by the migrating nuclei.

It will now be seen that the introduced nuclei can often be distinguished from the thallus nuclei in the ostiolar hyphae and at the base of the young aecium. It must be confessed, however, that the writer has not been able to recognize them in the vegetative hyphae leading from the pycnium to the primordium. Several factors combine to make this difficult. In the first place, the nuclei diploidizing a given primordium may often have entered through one of several pycnia. Secondly, the connecting hyphae do not run directly to the primordium, but meander so much that it is difficult to trace them from section to section. A third factor is the indistinctness, with the most useful cytological methods, of many of the septa; this makes it difficult to decide whether two nuclei are actually in the same cell or not. Finally there is the variability in appearance of the hyphal nuclei, which makes certain distinctions very difficult.

It is also suspected that the introduced nuclei pass through the connecting hyphae very rapidly, a behavior that would lessen the chance of finding them. There is a strong possibility that these nuclei divide in the receptive hyphae, as is suggested by their distorted appearance and by the presence of two or three of them in a single hypha while adjacent hyphae contain none. Such divisions would tend to retard their movements. It is probable, however, that no such divisions occur in the journey from the pycnium to the edge of the primordium. Since the ground mycelium through which they pass is certainly not diploidized and no signs of such divisions have been found, there appears to be nothing to delay them.

When the edge of the primordium is reached, the nuclei evidently divide very freely. Divisions have occasionally been seen at the extreme base of primordia in which diploidization was just starting (fig. 62, 63). It is not likely that the thallus nuclei in this position would undergo division. The point cannot

be tested, however, for although early stages of the division might be found and might prove to belong to the unexpanded nuclei, it is quite possible that the thallus nuclei in this region would be only partly expanded. Divisions of the diploidizing nuclei at the extreme base of the primordia are also suggested by the finding of several in a single cell, while adjacent cells of the same hypha contain none (fig. 85). It is improbable that nuclei of the same strain, and therefore mutually repulsive, would complete their migration in such close association instead of becoming widely scattered.

Complete diploidization of the primordium is apparently effected in several ways. It is started by the introduced nuclei continuing their passage through the hyphae by which they entered and pairing up with the expanded nuclei of the enlarged cells toward their apices. Several cells of such a hypha may be diploidized (fig. 86), and those deep in the primordium then bud out and grow up beside those nearer the apex, so that all the spore mother-cells are cut off at approximately the same level. Thus one may partly account for the intertwining of the basal cells in the mature accium that makes the origin of the dikaryon so difficult to determine. This could give complete diploidization only if large numbers of nuclei entered the primordium at many different points, for the primordium is made up of a number of hyphae that separate long before reaching it.

Actually, diploidization seems to start at one or two points only, and subsidiary methods therefore are needed. One method that is certainly adopted is the migration of extra nuclei from cells such as that at the upper right of figure 85 to cells in adjacent hyphae. Thus one of the introduced nuclei in the cell just referred to might very well migrate into the monokaryon cell directly below it. Such migrations would certainly be through narrow pores, for as long as the nuclei remain only partly expanded, they can easily revert to the unexpanded form, as when a haustorium is formed; and once the migration is completed, the point at which it occurred cannot be seen. These two methods of diploidization probably account for most of the nuclear migrations of the type first described by Blackman (1904).

A third probable method, the existence of which is difficult to prove or disprove, might be regarded as an extension of the one just described. If the cell to be diploidized was not actually touching the one from which the diploidizing nucleus was to be supplied, it is reasonable to suppose that a fusion papilla would be formed to bridge the gap. If, however, all the adjacent cells had already been diploidized, either all of these supernumerary nuclei would have to be functionless, or, instead of short papillae, one or more hyphal branches would have to be formed from the cell, and the introduced nuclei would have to move up in them to diploidize cells further from the base of the primordium. Two findings support this hypothesis. One is the occurrence of monokaryon cells with unexpanded nuclei (fig. 91) at about the level of the basal cells, after the start of diploidization when all the thallus nuclei in this region normally are expanded.

The second finding supporting the formation of such branches is the occurrence of equal-cell fusions such as Christman (1905) first described. There has been a tendency, since the discovery of the function of the pycniospores, to discredit either the reality or the importance of these fusions. Andrus (1931), in particular, has attempted to dismiss them as mere vegetative fusions that occur before diploidization. There can be little doubt, nevertheless, that diploidization does occur to some extent by this method in many rusts. In Uromyces Fabae these fusions are seen infrequently but rather regularly in the apical part of the basal cell region. This region is always filled by pseudoparenchyma tissue before diploidization starts, so that the vertically oriented fusion cells must be of later origin than the supposed vegetative fusions. That the fusions of vertically oriented cells are really functional is indicated by the occasional finding of fusions which result in the cutting of trinucleate spore mother-cells (fig. 95). Several examples, corresponding very closely to those described by Fromme (1912) for Melampsora Lini, have been seen in U. Fabae; it is sometimes possible to see that they have arisen by the fusion of a dikaryon hypha with a monokaryon. The evidence provided by such irregular fusions leaves no valid reason for denying the reality of the more typical ones.

It is probable that the nuclei of the introduced strain are often expanded by the time such fusions occur, and since the expanded nucleus does not seem to be so well adapted to passing through a small pore as the unexpanded one, one would expect either migration through a wide pore or complete fusion of the upper parts of the cells. The migration of an expanded nucleus through a relatively wide pore, about  $0.6~\mu$  in diameter, is shown in figure 90. Whether such a pore would remain this size or whether it would gradually enlarge to give a typical fusion cell remains in doubt.

One other possible method of diploidization has been suggested by certain findings in the young aecium. Cells are occasionally found that contain an odd number of nuclei in various stages of development. Lamb (1935) seemed inclined to regard such discoveries as due to excessive application of pycniospores in artificial mixing of the nectar. The situation shown in figure 89 is not satisfactorily explained in this way. Here is a well-established basal cell with its nuclei in telophase. The cell shown below it is probably a second basal cell, since only two daughter nuclei of a spore mother-cell, namely those entering the new spore, become expanded. In this cell there are three expanded nuclei and what is evidently another nucleus in telophase. That so many nuclei should have migrated into this cell while only one remained in the cell through which they came seems improbable. The most feasible explanation is that this is a normal basal cell in which conjugate division had just taken place, and that one of the nuclei had been stimulated by the proximity of an adjacent monokaryon cell to undergo a further division, which would supply a nucleus to the latter. Similarly the small dense nucleus of the trinucleate terminal cell of figure 91 may have been formed by the division of one of the paired nuclei in this cell, providing for the diploidization of the adjacent monokaryon cell. In this instance, as was noted above, the unexpanded form of the nuclei in this monokaryon hypha suggests that they are of the introduced strain, so that the dividing nucleus would be of the thallus strain. The small nucleus seen in figure 87 is apparently in late prophase and is probably dividing in preparation for supplying a nucleus for an adjacent cell.

The division of one nucleus of a dikaryon cell without division of the second is not unprecedented. It occurs extensively during the progressive diploidization of a haploid hymenomycete mycelium, as shown by Buller (1930), and it presumably occurs to some extent in the diploidization of a haploid rust pustule by a diploid mycelium, as described by Brown (1932).

Sometimes, admittedly, nuclei are seen that have no obvious function. For example, in the left hand cell of figure 88 a compact nucleus is seen close to a pair of nuclei in prophase. A small projection of chromatin at one side of it suggests that it was either about to migrate at the time of fixation or had just done so, but its function is not clear, and it is possible that this actually is a superfluous nucleus.

The cytological evidence that the introduced nuclei divide freely to diploidize the primordium is strongly supported by the hybridization experiments of Newton, Johnson, and Brown (1930), who crossed various physiologic forms of Puccinia graminis Tritici. They found that if the pycnial nectar of several forms were mixed in a pycnium, different forms of the rust resulted from the different aecia, but that all the spores of a single aecium were usually identical. This highly important finding clearly suggests that the diploidizing nuclei in an aecium are typically the product of a single pycniospore. This does not necessarily mean that only one nucleus reaches the primordium. It is possible that the nuclei divide after entering the receptive hyphae and that two may reach the primordium by different routes. As already mentioned, diploidization has been seen to start in two different parts of the primordium. It would seem that the primordium's power of attraction is greatly diminished as soon as diploidization starts, so that only the practically simultaneous arrival of nuclei at different points can give rise to an accium whose spores are not all genetically alike.

The aecium.—Conjugate division of the fully expanded nuclei starts in some of the basal cells and even spore mother-cells before diploidization of the primordium is complete. These conjugate divisions have been followed in some detail with the aid of the Feulgen method, which seems to be the only satisfactory means of staining the dividing nuclei without interference from the abundant ergastic material.

The start of prophase is indicated by the chromatin becoming gathered to one side of the ectosphere, giving an effect reminiscent of the "bouquet" stage in the meiotic prophase of many animal cells. At first the nuclei tend to appear as identical images (fig. 96, 97). Later, the chromatin masses come to lie in adjacent positions, making the nuclei appear as mirror images, as is illustrated by the right hand cell of figure 88 and the middle cell of figure 92. Intermediate stages, of course, are seen in which no relation is apparent. From the first appearance of the mirror-image condition there is considerable variation in the appearance of the various stages, because the time at which the ectosphere membrane and the endosphere break down varies widely. The chromatin of each nucleus next resolves itself into four threads with a maximum length about half that seen in the fusion nucleus; that is to say, the largest chromosomes attain a length of about 4  $\mu$  and the shortest ones about 2  $\mu$ . In the left hand cell of figure 88 the situation is complicated by the presence of a third nucleus, but this cell offers a perfect example of the mirror-image effect already mentioned. The two nuclei are separated by a very fine film of cytoplasm. Just to either side of this film can be seen one of the long chromosomes. Beyond this, and approximately parallel to it in each nucleus, lies the second long chromosome, a little more widely separated from the first in the left hand nucleus than in the right. Toward the apical end of each nucleus is seen one of the short chromosomes, curved and lying at an angle to the long ones. The second short chromosome slightly overlaps the first and lies nearly parallel to the long ones. These mirror images clearly indicate that the dikaryon condition is preserved by two opposing forces acting on the two nuclei.

In the pair of nuclei just described the ectosphere has apparently disappeared, or, at any rate, is in the process of breaking down, and the endospheres have already disintegrated. Usually the endospheres persist until later in the division. The central cell in figure 88 shows a later prophase stage with the chromosome contraction in progress. The mirror-image effect is not clear, because the nuclei were not in the same focal plane. The endospheres are still intact, though one of them has drifted off into the cytoplasm after the dissolution of the ectosphere membrane. Metaphase figures are not easily recognized, because the spindle is not clearly shown with the Feulgen stain, but early anaphases are found without difficulty. If the ectosphere membrane is still intact, the spindle forms as a chord to it (fig. 98); but frequently it disappears before this stage, as in figure 99, where one of the endospheres has also disappeared, while the last remnant of the other is visible.

The direction of the spindles, relative to each other and to the cell, seems at first to be a matter of chance, both in the basal cell and in the spore mother-cell (fig. 92). This may be true until early telophase (fig. 100); but usually by this stage the spindles lie side by side, parallel to the long axis of the basal cell (fig. 89) and diagonally in the spore

mother-cell (fig. 93), where the intercalary cell is cut off somewhat to one side of the spore chain (fig. 101).

In late anaphase and early telophase we often see the effect (fig. 89, 92) that led Sappin-Trouffy to postulate a haploid chromosome number of two for the rusts. As the telophase progresses, the new endosphere is cut out (fig. 94) in the way described for the urediniospore, and the chromatin becomes distributed over its surface as in the top cell in figure 93. The pairs of nuclei resulting from the division of the basal cell both assume the expanded form; but of those resulting from the spore mother-cell division, only the spore nuclei become expanded, while most of the chromatin of the intercalary cell nuclei remains within the endosphere.

It is of interest to note (fig. 101) that the intercalary cells of a spore chain are generally cut off toward the same side. Possibly this has some function in the mechanism of spore discharge, but it seems more probable that it is connected in some way with the development of this unique method of spore production.

The spore nuclei are usually fully expanded by the time the spores are fully grown. The chromatin in the mature spore varies greatly in distinctness. Sometimes it is nearly all situated close to the ectosphere membrane and is not very evident, being, apparently, very finely dispersed. At other times it forms a distinct reticulum, but the heavy reticulum often obtained in haematoxylin preparations (fig. 103) is never duplicated with the Feulgen stain. This further indicates the misleading effects that may be obtained with dyes not truly specific for chromatin.

The germination of the aeciospores, including reversion of their nuclei to the unexpanded type, is identical with that already described for the urediniospores.

Here and there in the aecium of *Uromyces Fabae* one finds chains of columnar cells (fig. 102) with slightly expanded nuclei. No intercalary cells are formed in these chains, the cells remaining undivided after being cut off from the basal cells. These cells are thus the morphological equivalent of the spore mother-cells and of the peridial cells, the final division of which is also deleted. It should be noted that the basal cells from which these cells are cut off have the same type of nucleus. They may simply be an abnormality, but it is quite possible that they serve a purpose similar to the buffer cells in the uredinium and telium.

Finally, a few words should be said about the peridial cells, for they clearly illustrate the nuclear condition of the vegetative cells in general. They have nuclei that are actually slightly expanded, but this is not very apparent with some staining methods. To illustrate this point three lateral peridial cells of Uromyces Fabae are shown in figures 104, 105, and 106. Figure 104 was stained with haematoxylin and figure 106 with the Feulgen stain. It will be seen that the nuclei of both cells appear uniformly reticulate, no endosphere being discernible. Figure 105 was

stained by the buffer method. The endospheres are very distinct and are about 1.25  $\mu$  in diameter, while the nuclei of figures 104 and 106 are about 2.0  $\mu$  in diameter. The outer part of each nucleus in figure 105 is virtually invisible because of the almost complete absence in these cells of the ergastic material on which the staining method depends. The abnormally small amount of this material present in the endospheres accounts for their invisibility in the haematoxylin preparation. It will be noticed that a small granule, surrounded by a "heller Hof," is visible in the endospheres in figures 103 and 105. This may possibly be a true nucleolus.

Discussion.—The changes in nuclear form here described for the rusts are decidedly unusual, and both the mechanisms involved and the causes underlying the changes are in need of explanation. It is to be hoped that further study in this group and, particularly, in other groups of the higher fungi will make the complexity of the rust nucleus more comprehensible. In the meantime the existence of these changes must be faced, and we must interpret them as best we can.

We must first consider how the whole of the chromosome substance can pass through the endosphere membrane, apparently without this membrane becoming in any way disorganized. The passage of chromatin through a membrane was first postulated in Hertwig's (1902) chromidial hypothesis. Cowdry (1924) discussed this hypothesis fully, pointing out that the term chromatin has all too often been applied to any substance that shows an affinity for basic dyes; but he concluded: ". . . . we are obliged to admit that it is by no means a rare occurrence for nuclear material to pass into the cytoplasm in large or small amounts." In the rusts we have a refinement of this situation. The nuclear material passes not into undifferentiated cytoplasm, but into an outer nuclear sphere, the ectosphere, newly formed from the cytoplasm. Modern microdissection investigations help to explain the situation. As Dr. Robert Chambers pointed out to the writer several years ago in connection with a different problem, a cytoplasmic or nuclear membrane, if violently pierced, will rupture; but if a needle or other object is gently introduced, no damage is done, and the membrane reforms behind it as it is withdrawn. The membrane, after all, is not to be regarded as a solid structure, but rather as a layer of oriented molecules, perhaps of lecithin or some related substance, at a liquid-liquid interface.

The problem of the functional necessity for the development of two distinct nuclear types is really more important than the mechanism involved in the change. One striking feature is that the unexpanded nuclear form occurs at every point of the life-cycle where the nuclei must pass through a very small pore. These stages are: the quadrinucleate basidium, the nuclei of which pass through the narrow tip of the sterigma; the basidiospore, which infects by the formation of a slender thread passing into an epidermal cell; the intracellular hypha resulting from

basidiospore infection, the nuclei of which pass through a narrow pore into the intercellular space; haustorium formation, during which the nuclei enter the host cell through a narrow pore; the pycniospore, the nucleus of which passes into a receptive hypha; and finally the migrating pycniospore nucleus, which passes through the septal pores on its way to the primordium. It should further be noted that Pady's (1935b) illustrations of the persistent intracellular mycelium of Kunkelia nitens indicate what may be the same situation; the endosphere is evident only in the intercellular hyphae.

The only other points at which the nuclei are typically unexpanded are the brief interphase in the basidium, and the germination of the aeciospore and urediniospore. The germ-tubes of the aeciospores and urediniospores generally enter the host through the stomata, and their nuclei may divide several times before any haustoria are formed. The only recorded exception to this behavior is found in the aeciospores of Gymnoconia Peckiana. These were shown by Pady (1935a) to enter directly through the epidermal cells, but even here nuclear division usually preceded infection. The germ-pores of the aeciospores and urediniospores are always fairly large, and either type of nucleus can be formed, indifferently, after mitosis; the complicated process by which these nuclei discharge their endospheres does not, therefore, seem to have any advantage unless penetration of a host cell is to precede nuclear division. Then it would be functional, since apparently only the partly expanded nucleus, and not the fully expanded one, can revert to the unexpanded form by the method used in haustorium formation. Whether such a type of infection occurs in any exist-

ing species is doubtful.

In the fusion nucleus the prophase chromosomes attain such a length that the very large ectosphere might be essential here if these long chromosomes are necessary for the unrestricted formation of chiasmata. This, however, explains the very similar expansion in the aeciospores and urediniospores, and in the basal cells and spore mother-cells of all three stages, only if one assumes the evolution of all three types of sorus from a simple telium in which the spores were formed directly from hyphal cells without the intercalation of basal cells and spore mothercells.

Mechanical advantage may, conceivably, not be the sole explanation of the change in form of the nuclei. It will be noted that the expanded nucleus is found in those spores that must make extensive growth before renewing nutritive contact with the host. Acciospores and urediniospores frequently produce germ-tubes exceeding  $500~\mu$  in length, and their nuclei may divide several times before a host cell is entered. It is not known to what extent the germinating teliospore is dependent on the thallus to which it commonly remains attached. It appears from the work of Dietel (1915) that the water needed for germination is absorbed through the pedicel; but when the host tissue dies in the fall and the

spore does not germinate till the following spring, it seems likely that all the food needed for the production of the basidium and spores is stored within the teliospore. Possibly the reserve nuclear material is largely stored within the nucleus. In sharp contrast to these spores are the basidiospore and pycniospore, which have unexpanded nuclei and also make little or no independent growth. The basidiospore puts out a short beak before passing directly into a host cell, and the pycniospore nucleus migrates into a receptive hypha of the thallus without any true growth occurring.

Even if food storage has no bearing upon nuclear structure, it certainly affects the staining reactions of the nuclei. A study of the staining reactions suggests a well-ordered economy in the protein metabolism of these highly specialized parasites. It has been observed, by using a number of staining methods, that there is a strong tendency for the rust nucleus to stain like the host nucleolus and for the rust cytoplasm to stain like the reticulum of the host nucleus. A lack of the necessary enzymes for the elaboration of proteins from simpler substances may partly explain the obligate parasitism of the rusts. These staining reactions, coupled with the close association of the haustorium with the host nucleus observed by most students of the group, suggest that the host enzymes may serve to break down the host proteins into amino-acids and that these, after absorption by the haustorium, are once more elaborated into proteins with the aid of the host's own enzymes. It is not to be assumed that the resulting proteins would be identical with those of the host, since it is well known that a slightly altered environment will materially affect the products of enzyme activity. It has been observed that both the nucleoprotein and the other stainable proteins of the nucleus appear, from the staining reactions, to be greatly diminished in the invaded areas of a rusted leaf.

It has already been stated that the nuclei of the basidiospores and pycniospores are scarcely distinguishable with the buffer stain, while those of the thallus and the other spore stages stain strongly. The unusual phenomenon of the nucleus taking an acid dye in preference to a basic one is explained by the fact that it is ergastic material, probably mainly proteins related to those found in the host nucleolus, that is stained rather than nucleoprotein. By checking with the Feulgen stain it can be seen that much of the nuclear substance stained with haematoxylin is also not nucleoprotein, and this is probably the same material. The basidiospore and pycniospore, as already pointed out, need little reserve material, and this accounts for their poor stainability. It is significant that the staining reactions of the ostiolar hyphae suggest that they contain more of this material than do the vegetative hyphae. This is evidently an economy on the part of the fungus, since any ergastic material in the hyphae can be retrieved for use elsewhere in the thallus, but most of that in the pycniospores must be lost because very few of them succeed in performing their function.

It is further to be remarked that the staining reactions show that much of the ergastic material in cells that have performed their function or that have only a mechanical function, such as the peridial cells, is removed for use in the more active cells.

Unfortunately very little detailed information is available on nuclear structure in the other groups of the higher fungi. A study of the published figures suggests that, with the exception of the fusion nucleus, the nuclei of the Hymenomycetes correspond to the unexpanded type of nucleus in the rusts. The writer has been fortunate in having the opportunity to examine many of Dr. Alexander H. Smith's preparations of various species of Mycena. It is evident that in this genus the paired nuclei in the young basidium are of the unexpanded type, that expansion starts just before or just after nuclear fusion, and that the expansion is a process of vacuolization essentially similar to that seen in the rusts.

The extensive cytological studies of the Ascomycetes have been directed along lines very different from those followed in the present investigation, and interpretation of the nuclear structure is generally impossible. Two recent papers, however, suggest points of interest. DeLameter (1937) shows the hyphal nucleus of Eidamella spinosa as a dark sphere about 1.5  $\mu$  in diameter, in a hyaline zone about 2.0  $\mu$  in diameter. This strongly suggests a similarity to the slightly expanded nucleus commonly found in the more active rust hyphae. His division figures indicate that division starts within this inner sphere rather than beside it in the outer cavity.

Colson (1938), in an extensive study of Phyllactinia corylea, maintains that the binucleate condition of the oogonium arises by division of the oogonium nucleus rather than by the entrance of the antheridial nucleus. It is difficult to secure an exact picture of these nuclei from her somewhat conventional figures, but one nucleus is large and has a large inner body; the other is much smaller and bears some resemblance to the unexpanded rust nucleus. A comparison of Colson's text figure 7 with the writer's figure 85 will emphasize this similarity. A point stressed by Colson was that the proportion of ascogenous cells that formed asci was so high as to preclude the possibility of nuclear migration and the existence of an irregular dikaryon; for the divisions in the ascogenous hyphae were not conjugate. By "conjugate" Colson evidently meant side by side rather than simultaneous. If, however, the divisions occur simultaneously but end to end as in the dikaryon hyphae of the rusts and if the nuclei subsequently slide past one another, the high proportion of compatible pairs could be obtained.

The mechanism by which the nuclei of the dikaryon cells maintain their relationship as pairs has always been a matter of interest. The mirror-image effect seen at prophase in the aeciospore basal cells clearly indicates the existence of two opposed and balancing forces. The mutual repulsion might well be due to similar charges on the two nuclei. Certainly it must be a force that will be increased as the nuclei are brought closer together; otherwise a balance could not be effected. It must also be due to some quality shared by all nuclei rather than to a difference between the nuclei of opposite strains; otherwise the balance seen in the occasional trinucleate cells could not be maintained. The mirror effect seen in figure 88 indicates that one of the forces acts upon a particular point in each chromosome. If the repulsive effect is due to equal charge, it is necessary only to assume that the charge is reduced at the time of fusion, rather than reversed, for the force of attraction will still be active. The occurrence of trinucleate cells is of additional interest, since it indicates that, even though the nuclei of a haploid thallus are alike in strain, there may be a difference in potency between sister nuclei. This may account for occasional findings that seem to represent the migration of a nucleus from a dikaryon cell to a monokaryon.

The present observations seem to strengthen the view that the process of diploidization should be regarded as essentially similar to the process in the Hymenomycetes. The attempt to identify the structures involved as male and female organs leads to

serious difficulties.

Lamb (1935) pointed out that, according to the view that the receptive hyphae are trichogynes leading to egg-cells, diploidization by the fusion of hyphae from intermingling monokaryon mycelia is fertilization of one female element by another. Ashworth (1935b), discussing the stomatal hyphae accompanying the pycnia of Endophyllum Sempervivi, pointed out that connections could be traced between these hyphae and others leading to haustoria, whereas a trichogyne would be expected to connect only with a reproductive structure. The same investigator (1935a) described the occurrence of stomatal hyphae in different stages of several rusts. The finding of such hyphae accompanying the uredinia of Uromyces Fabae strengthens Ashworth's contention that they must not be regarded as specialized female structures, although it is possible that they occasionally serve as receptive hyphae.

In the species examined it seems certain that the ostiolar hyphae of the pycnium are the main, if not the only, places of entry for the pycniospore nuclei. This militates against the view that the pycnium is

strictly a spermogonium.

It is evident that few introduced nuclei reach a primordium and that its diploidization is completed by these few dividing repeatedly to supply the required number. This fact and others gleaned from a study of the partly diploidized accium show the behavior of the pycniospore nuclei to be very different from that of the nuclei of true spermatia, which do not divide repeatedly during their migration to the egg. They do not divide, after entering the egg, to supply spermatia for adjacent eggs; nor do we expect to find eggs arranged in rows, so that the spermatia have to pass through one in order to reach another. Finally, it is seen that the difference in size and form between the nuclei of the thallus and the

introduced nuclei is not one of sex: if this were so, the nuclei of the basidiospores and haustoria would be male, and those of the aeciospores and uredinio-

spores female.

When the differences in mode of life of the Hymenomycetes and the rusts are considered, it is no longer surprising that their diploidization processes should have developed along somewhat different lines. It must be remembered that the rust thallus soon virtually exhausts the available food material in the invaded part of the leaf or stem. This condition would impose a serious handicap upon the pycniospore if it had to grow through the leaf to the primordium. Lacking large quantities of reserve material, the pycniospore would have to establish connection immediately with the host or with the thallus. If the host cells are already exhausted, the logical solution of the difficulty is for receptive hyphae to be formed so that the pycniospore nucleus can enter the thallus without penetrating the host at all. The production of nectar in the pycnia for the attraction of insects that will distribute the spores entails the production of receptive hyphae where the insects will deposit these spores-namely, in other

Bacteria and fungi cannot normally grow in the nectar. (That they do occasionally grow in diluted nectar if the weather is very moist may explain some reports of pycniospore germ-tubes growing down through the ostiole.) As Dr. A. H. R. Buller pointed out to the writer some time ago during a discussion of this subject, quite apart from the need of preventing mold growth in the nectar it is necessary that the pycniospores should be unable to grow in the nectar of their own pycnia. What could be more natural than that, having developed a method of entering the thallus without germinating, they should in time lose their power of germination? Parenthetically it may be suggested that some of the cases of slight germination reported for these spores may actually be merely the effect often seen when bacteria are placed in strongly hypotonic solutions: the protoplast swells to such an extent that it bulges out through the weakest part of the wall.

The invading nuclei do not diploidize the cells through which they pass. As Lamb pointed out, to do so would be waste of nuclear substance; for the future growth is localized in the aecium, and the remainder of the thallus serves only to conduct nu-

trient material to it.

The acceptance of the view here expressed necessitates the use of the terms "pycnium" and "pycniospore" in preference to "spermogonium" and "spermatium." It is unfortunate that Lamb, who strongly championed the same view, should have obscured his argument by clinging to the latter terms.

## SUMMARY

By the use of new staining methods to supplement the usual procedures it has been shown that there are two distinct types of nucleus in the rusts, and the transition from one to the other has been elucidated. What is termed the unexpanded form is adopted in every part of the life-cycle where migration of the nucleus through a narrow pore is necessary. In the transformation of the unexpanded nucleus into the expanded, a new nuclear sphere, the ectosphere, is formed about the original nucleus. The chromatin passes through the original nuclear membrane and becomes distributed through the ectosphere, leaving the original nuclear sphere, or endosphere, completely devoid of it. The endosphere is the body commonly referred to by rust investigators as the nucleolus, but the use of this term is to be deprecated, since it is not homologous with the nucleolus of the higher plants. What is possibly a true nucleolus may sometimes be discerned within it.

The expanded nucleus is found in the aeciospores, urediniospores, and teliospores, and in their basal cells and spore mother-cells. This suggests the possibility of a common origin for these three spore forms.

With the aid of the Feulgen method, nuclear division has been followed in greater detail than was

previously possible. In the unexpanded nucleus the spindle forms equatorially in the single nuclear sphere. In the expanded nucleus it forms beside the endosphere as a chord to the ectosphere membrane.

The nuclei of the mycelium usually have some of their chromatin outside the endosphere, but their division is essentially similar to that of the unexpanded nuclei.

Evidence has been secured that, in *Puccinia Sorghi* and *Uromyces Fabae* at least, the pycniospore nuclei enter the thallus through the ostiolar hyphae of the pycnium and that very few of these nuclei enter each primordium. Complete diploidization is accomplished by their repeated division after the primordium has been reached.

Preservation of the dikaryon condition by the operation of two opposed, balancing forces is indicated by the observation that in prophase the conjugate nuclei in the accium tend to appear as mirror images of each other.

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# A DEVELOPMENTAL ANALYSIS OF HETEROSIS IN LYCOPERSICON I. THE RELATION OF GROWTH RATE TO HETEROSIS 1

# William Gordon Whaley

Observations on the occurrence of hybrid vigor were a part of the first studies of plant hybridization. Kölreuter (1766), Knight (1799), Sagaret (1826), and Gärtner (1849), among the early investigators, recorded instances of great increases in size and yield in the progeny of certain crosses. Mendel (1865) reported that progeny secured in crossing two types of Pisum usually exceeded either parent strain in stem length and showed greater luxuriance generally. Nearly all the early papers Received for publication April 24, 1939.

The writer wishes especially to express his appreciation to Professor E. W. Sinnott under whose direction this investigation was conducted. He is also indebted to the members of the Department of Botany of Cornell University and to Professor R. A. Emerson for the use of laboratory and field facilities in the summer of 1938 and for much helpful criticism, and to Professors Eric Ashby and E. W. Lindstrom for their kindness in supplying seed

The second paper in this series will appear in a later issue of the American Journal of Botany.

contain references to occurrence of greater vigor in the progeny of crosses, but no attempt was made to explain the phenomenon until many years later when Jost (1907), largely on the basis of Darwin's (1875, 1877) observations, suggested that the more vigorous growth of hybrids has as its cause the same stimulus which enables the egg to develop.

It was, however, some time after the discovery of Mendel's paper before attention was focused upon the immediate effects of cross-breeding. An understanding of Mendelian principles of inheritance resulted in several attempts to explain the cause of in-

creased vigor in hybrid plants.

G. H. Shull (1908, 1909, 1910) and East (1908, 1909), working independently with maize, established the main facts upon which the first explanations were based. Both observed that self-fertilization in maize led to a decrease in size and vigor, the decrease becoming less and less in each generation until eventually a point was reached beyond which (presumably) no further loss of vigor took place. They noted further that progeny derived from crossing inbred lines usually excelled the parental types and that the yield per acre from the  $F_1$  generation was always greater than that from the  $F_2$  generation. After observing a large number of crosses, Shull (1910) suggested that vigor of hybrids is a direct result of hybridization and that the degree of vigor is directly dependent upon the number of heterozygous factors present.

Keeble and Pellew (1910) reported a cross between two half-dwarf varieties of Pisum which produced a hybrid greatly exceeding either parent in vigor. They noted that one of the parents was characterized by long internodes and thin stems and the other by short internodes and thick stems. On the basis of the F2 distribution they assumed one parent to have the genetic constitution tL and the other Tl, these being factors controlling stem diameter and internode length, respectively. The hybrid, of course, has the genetic constitution TtLl, and the authors ascribed the increased vigor to the presence of both of these factors. In the same year Bruce (1910) published a mathematical interpretation supporting the idea of combination of simple dominants as the cause of hybrid vigor.

In 1912 East and Hayes published the first paper devoted exclusively to the phenomenon of hybrid vigor. The authors reviewed G. H. Shull's data (1908, 1910, 1911) and their own previous work (East, 1908, 1909; Hayes and East, 1911). Both Shull and East and Hayes noticed that the degree of increase in vigor secured from a particular cross varied directly, at least in most cases, with the degree of heterozygosity which the cross produced. Since the increase in vigor is associated with hybridity, and hence with heterozygosity, and as the degree of increase appears to depend directly upon the degree of heterozygosity possessed by the hybrid, they assumed that heterozygosity itself is the immediate cause of hybrid vigor.

In 1914 G. H. Shull restated this hypothesis. He pointed out, however, that while the degree of hybrid vigor present seems to depend, within limits, upon the genetic dissimilarity exhibited by the parents, the combination of different Mendelian genes may be only one factor concerned. There remains also the possibility of the effect of the elements of the male nucleus upon the egg-cytoplasm, as pointed out by A. F. Shull (1912a, 1912b). For the purpose of avoiding the implication that all the genetic differences responsible for the stimulation of physiological processes in hybrids result from gene action, Shull proposed the term "heterosis" as a substitute for the phrases "stimulus of heterozygosis," "heterozygotic stimulation," "stimulating effects of hybridity," and other terms previously used to denote the condition bringing about increased vigor in hybrids.

Castle (1916) made a study of the growth of guinea pig hybrids and their parents and found evidences of hybrid vigor. He drew a distinction between the direct effects of inherited characters upon the size of the offspring and the effect that a combination of different gametes has upon size through increasing metabolic activity and supposed that a chemical difference between gametes caused the added vigor.

In 1918 Jones proposed a new explanation for heterosis by assuming the action of linked, dominant genes. Jones' hypothesis rests upon a series of assumptions: first, that there are many genes concerned in the development of any of the important characters of an organism; second, that these genes belong to various linkage groups; and third, that the genes concerned with the development of greater size are, at least partially, dominant.

There is much evidence to support the first and second of these assumptions. Many genes must be involved in the complex processes which are part of development, and if a large number are concerned, they must be located on at least several if not all of the chromosomes. The third assumption, however, that genes concerned with the development of greater size are usually dominant over those for lesser size lacks proof. Most hybrids are intermediate with respect to commonly observed size characters, and rarely does there seem to be any dominance of large size. Without at least some degree of dominance, however, there would be no cumulative size effect in the heterozygous condition, and Jones' explanation would not hold.

Collins (1921) proposed another hypothesis which does not require the assumption of dominance. From observation of the effects of inbreeding in maize, he believed that the loss of vigor which it produces is due not to the elimination of dominant, favorable factors but to the appearance, in homozygous form, of deleterious recessives. Conversely, the increased vigor which so often attends hybridity Collins attributed to the suppression of these deleterious recessives.

Comparatively little attention was paid to the physiological basis of heterosis until the experiments of Ashby (1930, 1932, 1937) with maize and tomato hybrids. Ashby's conclusions may be summarized as follows: first, heterotic hybrids do not differ from their more vigorous parent as to cell size, growth rate, respiration rate, photosynthetic efficiency of the leaves, or time of flattening off of the sigmoid growth curve; second, the hybrids observed seem to inherit growth rates as dominant Mendelian characters; third, the hybrids have a greater initial embryo weight than the parental types. He assumes that if a hybrid does not grow more rapidly than its parents, and does not grow for a longer period of time, and yet is finally larger than the parents, it must have started with a size advantage. Since these conditions seemed to obtain in the material which Ashby observed, he postulated that hybrid vigor is due simply to the possession by the hybrid of a bigger embryo-more "original capital." The hybrid thus has an initial advantage which is maintained throughout the whole period of growth. Its larger embryo is in turn due to some very early developmental difference, either in rate or duration of growth. Luckwill (1937) has confirmed Ashby's results for tomatoes, working particularly with the later growth phases. He also made some sections through young shoot primordia in the seed and concluded that those of the F<sub>1</sub> plants are larger than those of the parents. Luckwill's results will be discussed in detail later in the second paper of this series.

Ashby's hypothesis has been attacked by several investigators. Sprague (1936) performed experiments with maize similar to those made by Ashby and obtained different results. He found that in the early (post-embryonic) growth stages the hybrids grew faster than the parents. Actually his results do not necessarily conflict with those obtained by Ashby, because they deal primarily with different parts of the growth curve. Ashby was concerned mainly with the grand period of growth, after the seedling stage, whereas the differences in rates which Sprague found were confined to the very early seedling stage.

Lindstrom (1935) attempted to test Ashby's hypothesis by cutting back growing hybrid seedlings. In this manner he sought to reduce the meristematic capital of the hybrids to the same, or a lesser, amount than that of the parents. In every case the hybrids finally exceeded the parents in both plant weight and yield. The growing tissue which Lindstrom removed, however, is not comparable to the "original meristematic capital" to which Ashby refers the phenomenon of heterosis.

East (1936) took Ashby to task both for his observations on the expression of heterosis and his theoretical deductions and then proposed a new explanation. He assumed that dominance is absent in size traits, but that genes controlling them occur in numerous series of multiple alleles. If in a given series each member affects a different physiological process, then the heterozygous condition may be expected to produce cumulative effects. That is, if a1 affects a somewhat different process than its allele a2, a1 a2 may have a greater effect than a1 a1 or a2 a2. This hypothesis implies that there is complementary action between different mutations at the same locus —that is, a<sub>1</sub> supplies what is lacking in a<sub>2</sub>, or vice versa. There is little experimental evidence, however, to support this idea of complementary action between alleles.

Until the publication of Ashby's papers dealing with the observation of physiological differences between inbred and crossed lines of maize and tomatoes the problem of heterosis was approached almost solely from the point of view of theoretical genetics. The effects of heterosis have been observed for the most part in mature plant characters, and interpretations have been concerned mainly with quantitative differences between hybrid and parental types. Little has been found as to the underlying manifestations of heterosis, and genetic interpretation has therefore been difficult. Ashby emphasizes, quite properly, that the causes of hybrid vigor must ulti-

mately be physiological in nature. The problem is a developmental one, and any understanding of it requires first a knowledge of the developmental differences concerned.

The present study deals with a series of experiments designed to determine the character and developmental basis of some of these fundamental differences. The problem has been approached through a comparative study of the development of inbred and hybrid plants, with particular reference to the rôle of the apical meristem. It was thought desirable to study this meristem in some detail, both because of Ashby's contention that the "original meristematic capital" is the principal factor concerned in heterosis and because the apical meristem, as the functional embryo of the aerial parts of the plant, is the seat of origin of morphological differences observable in mature plants and their organs.

MATERIALS.—Two different tomato hybrids and their parents were used as experimental material. For the sake of brevity, the types will be described in detail and thereafter referred to by designated symbols.

Cross I.—Race A of this study was Lycopersicon esculentum, Line 1755-7, secured from Professor E. W. Lindstrom. This variety is a dwarf type. The plants are relatively slow growing, rarely attaining a height of more than two feet. A heavy pubescence covers all the vegetative parts. The fruits, which are borne in a few dense clusters near the apex of the plant, are pear-shaped and yellow in color. Fruits, flowers, and leaves are all of medium size. The line is homozygous for the genes d, p, o, s, r, y (MacArthur, 1931).

Race B was Lycopersicon pimpinellifolium, Line 1748-5 (Lindstrom), a race of the "currant" tomato. The plants have long slender stems. Stems, leaves, flowers, and fruits are all small in comparison to the other species used. Many fruits are produced, but the number of seeds per fruit is small.

Hybrid A  $\times$  B [1755-7 (female)  $\times$  1748-5 (male)] was the hybrid from which most of the data were collected. A detailed description of this plant will be given later.

Hybrid B × A was not obtained in large enough numbers for experimental use, because the small size of the flowers of plant B made hybridization difficult and only a few seeds were produced in each fruit. Observation proved, however, that it does not differ from its reciprocal.

Cross II.—Race C was Lycopersicon esculentum var. "Chinaman." The seed were obtained from Professor Eric Ashby, who states that this variety has been cultivated by Chinese gardeners in Australia since about 1860. The line is homozygous for several recessive genes, including br, brachitic stem with shortened internodes; f, fasciated fruit; and wt, a gene producing a curious inrolling of the leaf margins. The plants rarely reach a height of two feet.

Race D was Lycopersicon racemigerum, a South American species which is homozygous for the genes D, P, O, R, S, Y of L. esculentum, or homologous

TABLE L.

	A	В	$A \times B$	$\mathbf{c}$	D	$C \times D$
Height, 14th week Weight, 15th week:	66 cm.	99 cm.	123 cm.	54 cm.	88 cm.	102 cm.
Fresh	450 gm.	940 gm.	1,500 gm.	472 gm.	900 gm.	1,018 gm.
Dry (see text)	188 gm.	235 gm.	461 gm.	210 gm.	555 gm.	622 gm.
Fruit:						
Number per plant	72ª	139	233	28	32	86
Average weight Total weight per	39.44±.22 <sup>b</sup> gm.	1.8±.01 gm.	$6.04\pm.07$ gm.	93.4±1.8 gm.	11.4±.11 gm.	34.1±.58 gm.
plant, 15th week	220 gm.	250 gm.	1,400 gm.	2,638 gm.	350 gm.	2,920 gm.
Corolla width	26 mm.	10 mm.	20 mm.	34 mm.	19 mm.	23 mm.
Leaf length	$24.83 \pm .41 \text{ mm}$ .	$13.8 \pm .36 \text{ mm}$ .	$21.25 \pm .27$ mm.	$24.21 \pm .21 \text{ mm}$ .	$23.3 \pm .27 \text{ mm}.$	$24.45 \pm .32 \text{ mm}$ .
Leaflet length Cell size, petiole pa-	$7.7 \pm .12$ mm.	$3.66\pm.09$ mm.	$6.6\pm.08$ mm.	8.32±.13 mm.	$6.93 \pm .13$ mm.	$7.23 \pm .21 \text{ mm}.$
renchyma (diam.)	$123.8 \pm 9.3 \mu$	$66.6\!\pm\!4.9~\mu$	$95.2 \pm 7.1~\mu$	$109.5 \pm 9.8~\mu$	$101.2 \pm 6.2 \; \mu$	$114.2 \pm 5.2 \; \mu$

a Immature fruits counted to determine total.

b Standard error.

genes. These seed were also secured from Professor Ashby. The plants grow ten feet or more in height when staked. The fruits, borne in large trusses, are about the size of gooseberries. The species is selffertile and is somewhat difficult to cross. These C and D races have been used by both Ashby (1937) and Luckwill (1937) for studies of hybrid vigor.

Hybrid C  $\times$  D [ $\dot{L}$ . esculentum var. "Chinaman" (female)  $\times$   $\dot{L}$ . racemigerum (male)] has been used for most of the observations. Its characters will be discussed in detail later.

Hybrid D $\times$ C, the reciprocal cross, was observed for some characters and found to be essentially the same as the hybrid C $\times$ D. It differs somewhat in seed size, and consequently minor differences are observable in the level of the growth curves during the early stages.

Hybrid seeds of these crosses were first obtained from Professors Lindstrom and Ashby, but each of the crosses has been repeated many times by the writer

Large numbers of plants of the parents and progeny of each cross were grown in the field for two summers, first in Amherst, Massachusetts, during the summer of 1937, and then in Ithaca, New York, during the summer of 1938. Plants were also grown in the greenhouse of Columbia University during two winters. The same results were obtained in each of the four seasons, so only those plants grown during the summer of 1938 at Cornell University will be discussed.

The life cycle of the plants was divided for study into three purely arbitrary stages: (1) the germination period; (2) the first eight weeks of growth; (3) the eighth to sixteenth weeks of growth. This division was made necessary by the writer's inability to make observations during sixteen consecutive weeks of field growth. The germination and very early seedling growth periods are treated separately because the plants were grown under different conditions.

For convenience the report on this investigation has been divided into two parts—the first dealing with a study of growth rates and size differences as related to heterosis, the second with the rôle of the apical meristem in heterosis. The second part will be reported in a paper to follow this one.

CHARACTERS AFFECTED BY HETEROSIS.—A knowledge of the characters concerned in the expression of hybrid vigor is essential to an understanding of heterosis. Table 1 presents a comparison of some of the more readily observable quantitative characters of parents and hybrids of the two crosses.

Height.—In both crosses height of the hybrid exceeds that of the taller parent. Height, however, is not a good criterion in this instance because in each case one of the parents is a dwarf type. Further, the number of lateral branches developed has a marked effect upon height in tomato plants as does the time at which flowering begins and many other factors. Tall-dwarf crosses were purposely selected for study because their use makes it possible to determine whether meristem size is related to heterosis or the action of the Dd gene.

Leaf size.—Leaf size in tomatoes is difficult to measure. It is even more complex in the present instance because of the possession by one of the types of peculiarly rolled leaflets. No attempt has been made to determine areas, but leaf length has been recorded, together with the length of the second basal leaflet. These measurements afford a rough approximation of leaf size. Generally, the hybrid leaves are intermediate in size between those of the parents, but they tend to be farther above the mean of the parents than do the other determinate organs measured. Occasionally the leaves are larger than those of the larger leaved parent.

Flower size.—Size of the flowers in the hybrids observed is intermediate between those of the parents. This condition is illustrated by corolla width measurements given in table 1. Heterosis does not affect size of the flowers but rather the number produced.

Cell size.—Cell size was measured in each of the types, using parenchyma cells in the petioles of mature leaves. The values are given in table 1 and indicate that the hybrid does not have significantly

larger cells in either cross.

Fruit.—Yield, to be considered in relation to heterosis, must be analyzed into its two components, fruit size and fruit number. The effect of heterosis in increasing yield, as may be seen in table 1, is one of increasing the number of fruits per plant. Individual fruit weights in the hybrid are intermediate between those of the parents. As a result, increased fruit number is necessary in the hybrid before it can equal the yield of the larger fruited parent, and a great increase is essential to raise the yield materially. In both crosses the yield of the hybrid is greater than that of either parent.

Weight.—As indicated in table 1, the fresh weight of the vigorous hybrids exceeds that of the larger parent in each case. Fresh weight was used as a measure of growth and an indication of heterosis throughout the experiments, first because it probably is the best measure of plant volume, with which heterosis seems to be primarily concerned, and second, because it is the only measure equally well adapted to both germination and early growth and to later stages where measurement is complicated by the pro-

duction of fruit.

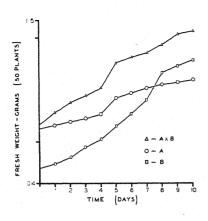
Facilities for making large scale dry weight determinations were not available, hence dry weights were determined only at intervals to furnish checks upon other measurements. The figures given in table 1 are average weights of plants air-dried for 14 days. The hybrid weight in each instance is greater.

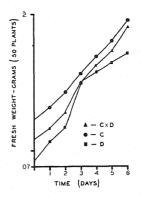
These results are in general agreement with what has been observed by other investigators. East (1936) noted that the effects secured from heterosis are very like those which might be obtained by adding fertilizers to the soil. This is certainly true in the present experiment. "Hybrid vigor" appears to be concerned only with total plant size. It seems to have little or no effect upon the determinate organs. Flowers, fruits, and even seeds are such organs, and they are not materially affected. Upon the leaves, however, there is some effect. It will be shown later that this is probably the result of the influence of heterosis in raising the general metabolic rate of the plant rather than its influence upon the size of the meristems or the leaf primordia. Flowers and fruits are end products. The leaf, on the other hand, is the primary metabolic organ of the plant. Hence, any change in the level of physiological activity might reasonably be expected to have an effect upon its size.

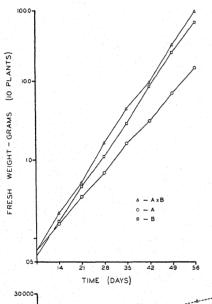
The relation of growth rate to heterosis.— Cross I (A, B, and A  $\times$  B).—Comparative growth rates during and immediately after germination were determined in the following manner: five hundred seeds of each type were selected individually so as to exclude waste material and injured seeds, and weighed. Seeds were soaked in water for four hours and placed on moist filter paper in damp chambers. They were arranged in alternate sectors on the paper, fifty seeds to a sector, six sectors to a dish. The seeds were about 8 mm. apart. The moist chambers were kept in an incubator at 28.5°C. At twentyfour hour intervals fifty seeds of each type were removed, dried lightly with soft absorbent paper, and weighed. Figure 1 shows the fresh weights of fifty plants, plotted on a log scale against time in days. The entry at zero time is the weight of fifty dry seeds. The first day includes the four hour soaking period. The first part of the curve undoubtedly represents only water absorption. Even in this period the hybrid and one parent (B) differ considerably from the other parent. The types seem to vary somewhat in the time when the initial marked increase in weight takes place. Germination in the hybrid is observable a few hours earlier than in either parent. The most significant point is the difference in order of the types in size at the end of the eighth day. The hybrid is still heavier, but the parent races have changed position, B having passed A. In view of the great difference between the seed weights of the two types, this result does not support the idea that the initial embryo size has an influence on growth, either as to rate or relative level. It is important that the general slope of the hybrid curve is essentially parallel to that of the faster-growing parent (B), but at a considerably higher level. What appears to be a decline in rate of growth during the later days represented in these curves is probably due solely to a reduction in food supply, since nothing except water was added to the cultures. This experiment was repeated four times with each set of materials, and the same results were obtained in each instance.

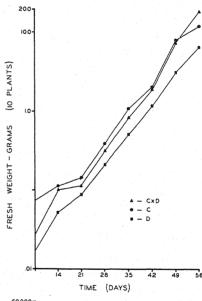
Growth during the second period, covering the first to eighth weeks, is shown in figure 2. Values given are total weights of ten plants in grams. The material used for this part of the experiment was planted in the greenhouse on July 1 and sampled at weekly intervals thereafter, being kept in the greenhouse during the whole eight weeks. The interchange of position by the parent types takes place a few days later in soil-grown plants than in seedlings grown in moist chambers. This condition is probably to be expected because of the more rapid early growth of the latter. The period of time represented in figure 2 covers the major part of the grand period of growth, after germination and before the setting of fruit. The results are in agreement with those of Ashby (1932, 1937) and Luckwill (1937) in that the hybrid has essentially the same growth rate as the faster-growing parent but is at a somewhat higher level.

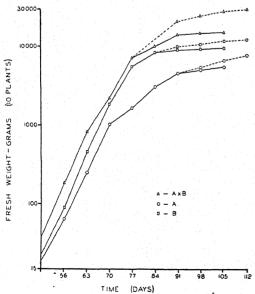
Figure 3 shows the fresh weight increase during the third period from the eighth to the sixteenth weeks. The plants represented here were planted in the greenhouse May 1, moved into the field June 20, and sampled weekly from the eighth to the sixteenth weeks after planting. Hence, while the plants of the second and third parts represent supplementary growth periods, and while all plants in each set were handled under uniform conditions, figure 3 must not

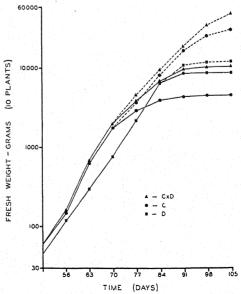












be considered as simply an extension of figure 2, because of the difference in environment. This does not, however, invalidate the results for comparative purposes. Fifty plants were weighed in the first five samples. As the plants increased in size it became impossible to handle such large numbers rapidly enough to prevent loss of weight by wilting, even though all sampling was done before sunrise. For this reason the number of plants was progressively reduced to forty, thirty, twenty, and finally ten in the last two sampling periods. In order that weights be comparable, the final figures have all been calculated on the basis of total weight of ten plants. The solid lines in figure 3 represent fresh weight of the vegetative parts of the plant; the dotted lines, plant weight plus fruit weight. Both sets of data show that the hybrid and its faster-growing parent (B) have about the same rate of increase. At approximately the seventy-seventh day the curves of the hybrid and parent B begin to level off, that of the parent a little faster than that of the hybrid. Growth begins to slacken in the smaller parent (A) about a week before it does in the larger parent and the hybrid. The curves indicate that the growth of parent A is very different from that of either B or A X B. Its fruits mature later, and for this reason had it been possible to grow the plants for a few more weeks, they might have produced more fruit; but since ripening was well advanced, it is probable that no significant increase would have resulted. Although the curves of both the hybrid and its faster-growing parent begin to level off at about the same time, that of the hybrid falls off less rapidly, and hence the difference between hybrid and parent is greater in the end than at any time during development.

"Hybrid vigor" in this particular cross is the result of three conditions: First, the hybrid has an initial advantage in that the seeds are larger. As will be shown later, this situation does not obtain in the other cross. Second, the hybrid gains more during the very early stages of growth, partly as a result of earlier germination and partly because it grows more rapidly. Thus the hybrid obtains an early advantage. As its growth rate during the grand period of growth is essentially the same as that of the faster-growing parent, this advantage is maintained. Third, the hybrid grows more than its parents during the final stages of growth. As a result, the initial advantage which the hybrid gained by faster early growth is markedly increased during the fruiting period, giving final differences greater than those at any time during growth.

Cross II.—The cross between L. esculentum var. "Chinaman" and L. racemigerum presents a somewhat different situation. The seed weight of the hybrid, the entry at zero time on figure 4, is intermedi-

ate between those of the two parents. If seed size of the hybrid is intermediate, presumably its embryo size is also. Luckwill (1937), however, reported that the initial dry weight of hybrid seeds in the reciprocal of this cross is significantly greater than that of either parent. The seeds of parent D begin to germinate slightly before the hybrid seeds.

Because of their larger size it was impossible to grow these plants in culture chambers as long as the plants in cross I. At the end of the sixth day, however, the seedlings occupy the same respective positions as to weight as did the seeds.

Figure 5 shows that while the hybrid growth curve is similar to that of parent C, during the grand period of growth it actually grows somewhat faster, since, after having started with a disadvantage in size, it exceeds the C parent in weight after about the seventh week. The entries in figure 6 indicate that here, also, the hybrid began to exceed the parent at about the same time. The change in slope of the curves between the 14th and 21st days in figure 5 is probably the result of retardation brought about by transplanting the seedlings.

Figure 6 shows the similarity between the growth curves of the hybrid and parent C, up to the time of fruit production. As in the previous cross, the curves begin to flatten off at about the same time. Here, also, the hybrid increases its advantage during the later stages. The increased advantage is more noticeable in the weight of the vegetative parts alone in this case, primarily because the hybrid had not completed fruit production at the conclusion of the experiment. The second parent, D, is of interest because it increases in weight much more slowly, but grows considerably longer than either of the other types, finally attaining an intermediate position with respect to plant weight. Its fruit yield is relatively low, perhaps because fruiting begins later.

Hybrid vigor in this cross, as in the first, is due to an early period of faster growth and to more active later growth in the hybrid. In this instance this faster growth is not so noticeable in the very early seedling stages but is seen mainly early in the grand period of growth. The hybrid starts out smaller than one parent in seed size, overtakes its larger parent at about the seventh week of growth, and gains an advantage which it again increases just before maturity. The amount of heterosis is less pronounced in the progeny from this cross than in the previous case, but it is clearly evident.

### SUMMARY

A study was made of growth rates of the parents and hybrids in two *Lycopersicon* species crosses showing heterosis.

Fig. 1-6.—Fig. 1 (above, left). Cross I. Increase in fresh weight following planting on moist filter paper. Cultures kept in the dark at 28.5°C.—Fig. 2 (center, left). Cross I. Increase in fresh weight for 56 days after planting. Plants grown in greenhouse.—Fig. 3 (below, left). Cross I. Increase in fresh weight from 49th to 105th days after planting. Plants grown in the field. Solid lines represent weight of vegetative parts; dotted lines, vegetative parts plus fruit.—Fig. 4 (above, right). Cross II. Same as fig. 1.—Fig. 5 (center, right). Cross II. Same as fig. 2.—Fig. 6 (below, right). Cross II. Same as fig. 3.

Heterosis, in the crosses observed, produces its effects by an influence on total plant size. The size of determinate organs is not increased by heterosis, but they are produced in greater numbers.

In both crosses the hybrid grows faster than either parent in the early post-embryonic stages and during the fruiting stages, but differences are much less marked during the grand period of growth.

The presence of heterosis is not always accompanied by the possession by the hybrid of a larger embryo.

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# HETEROTHALLISM AND SEGREGATION OF SEXES IN ASCOBOLUS GEOPHILUS <sup>1</sup>

Edwin M. Betts and Samuel L. Meyer

KNOWLEDGE CONCERNING heterothallism and homothallism in the Ascomycetes is, as yet, incomplete. Members of the genus Ascobolus Pers. furnish excellent material for investigations into the sexuality of the group. Dodge (1920) described heterothallism in Ascobolus magnificus Dodge, and his initial observation on sex segregation in the genus was confirmed by Gwynne-Vaughan and Williamson (1932). Heterothallism in Ascobolus carbonarius Karst. was demonstrated by Betts (1926), and it was shown for the first time that of the eight spores in an ascus, four are of one sex and four are of the opposite sex. Green (1931) found Ascobolus stercorarius (Bull.) Schröt. to be heterothallic, an observation confirmed by Dowding (1931). Schweizer (1931) described a new species, Ascobolus strobilinus Schweizer, which proved to be homothallic. Betts and Meyer (1938a) obtained results which indicate that sex segregation in Ascobolus carbonarius Karst. is not due to chance distribution. A preliminary report was recently presented by the writers (Betts and Meyer, 1938b) concerning sexuality in Ascobolus geophilus Seaver.

The importance of the Ascomycetes as material for genetic research has been clearly demonstrated through the investigations of Dodge (1928, 1931, 1934, 1935, 1936), Lindegren (1932, 1936), Lindegren and Lindegren (1937, 1939), Lindegren and Rumann (1938), Campbell (1937), Seaver (1937), and Dodge and Seaver (1938) and lends impetus to further studies of sexuality in the group. The present contribution is an elaboration of the preliminary report previously mentioned (Betts and Meyer, 1938b).

MATERIALS AND METHODS.—Ascobolus geophilus was first reported by Seaver (1911) as the European species, Ascobolus viridis Curr. It was later recognized as a distinct species and was redescribed as Ascobolus geophilus (Seaver, 1916). Its distribution is given by Seaver (1928) as New York to Iowa and Colorado and Winnipeg, Canada. Recently, in obtaining cultures of Ascobolus carbonarius Karst. from soil (Betts, 1926) collected at Charlottesville, Virginia, a single apothecium of Ascobolus geophilus Seaver appeared. The discovery of the species at Charlottesville extends the range and gives the first report of its occurrence in the South.

Spores from this apothecium were sown on soil decoction agar in sterile Petri dishes (Betts, 1926). The spores germinated within an hour without heating, a condition not found in other species in the genus, all of which require relatively high temperatures to initiate germination. After the mycelia from germinated spores had covered the surface of the agar, they were transferred in small blocks to sterile soil in jelly glasses, the soil having been pre-

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viously moistened with distilled water. Within three to four weeks mature ascocarps had developed. From these ascocarps spores were obtained in sufficient numbers to make possible an examination of the sexual strains in the species.

Spores from asci of Ascobolus are shot out with considerable force. If glass slides are placed over the apothecia, they are soon covered with spores. By means of microdissection needles, single spores were removed from the slides and placed in separate Petri dishes for germination. The mycelia were allowed to grow until the agar in the Petri dishes had become completely covered, and then blocks of agar were transferred to jelly glasses, as previously described. Eighty-nine monospore cultures, the spores selected at random, were numbered 1 to 89 and grown in triplicate, a total of 267 cultures.

To test the spores for plus and minus strains, fifteen monospore cultures were selected at random, labeled 1 to 15, and grown in all possible combinations. One hundred twenty crosses were set up in triplicate, a total of 360 cultures.

To determine the sexual strains of spores within a single ascus, apothecia were teased apart in water by means of finely ground needles, the single ascus picked up with a capillary pipette, placed on a glass slide, and allowed to dry. The ascus wall adhered to the surface of the slide, and it was then possible to remove the spores in the order of their occurrence in the ascus with the microdissection apparatus. Each spore was placed in a separate Petri dish of soil decoction agar for germination and the dishes numbered 1, 2, 3, 4, 5, 6, 7, 8, corresponding to the posi-

TABLE 1.ª

	 2	3	4	5	6	7	9	11	13	14	15	1	8	10	12
2	 											+	+	+	+
3				-					-			+	+	+	+
4			<del>-</del>									+	+	+	+
5		٠.			-					-		+	+	+	+
6	٠.		, · ·									+	+	+	+
7			•	• •	• •							+	+	+	+
9	٠.	٠,		• •		•,•						+	+	+	+
11	٠.	٠.	• •	٠.	• •		٠.٠		_			+	+	+	+
13	• •	• •	•		٠.	•	٠.	. • •				+	+	+	+
14	• •	• •	• •	. •	. • •	• •	• •		•••	7		+	+	+	+
15	• • •	• •		• •		•		• •	• •	••		, †	+	, +	-
1 8	• •		•			•,•,	•	• •	•••	• •	•	-			
10	•	• • •	٠	•	٠	* *	• •	• •	. • •	• •	•				-
12					• •	• • •	•	• •	• •	•	• •	•	•		
1~	• •	• •	• •	•		•	• •			• •		•		•	

<sup>&</sup>lt;sup>a</sup> The plus sign (+) is used to indicate the presence of apothecia resulting from the crossing of mycelia from individual spores; the minus sign (—) indicates that no apothecia were formed. Final observations were taken after a period of 30 days. A similar method of designation is used in tables 2 and 3.

tion of the spores in the ascus, spore 1 at the base and spore 8 at the apex. After germination, mycelia from the different spores were grown in all possible combinations.

A total of 706 cultures of Ascobolus geophilus was observed during the period of investigation.

DISCUSSION AND RESULTS.—None of the 89 monospore cultures produced ascocarps. This constitutes negative evidence proving that the species is not homothallic.

In table 1 are shown the results obtained when the mycelia from 15 spores, selected at random, were crossed in all possible combinations. Spores 1, 8, 10, and 12 are of one strain which may be arbitrarily termed the minus (-) strain. Spores 2, 3, 4, 5, 6, 7, 9, 11, 13, 14, and 15 are of the opposite or plus (+) strain. In no case were ascocarps formed when spores of the plus or minus strain were crossed with each other—that is, plus with plus or minus with minus. Apothecia appeared in all crosses involving mycelia from spores of opposite strain, as plus with minus or minus with plus. For example, spore 1 produced a mycelium from which no apothecia developed in crosses with the same mycelium or with the mycelium from spores 8, 10, and 12. On the other hand, when the mycelium which developed from spore I was crossed with that from any of the spores other than 8, 10, and 12, ascocarps were produced. Conversely, the mycelium from spore 2 produced no apothecia when crossed with itself or with the mycelium from spores 3, 4, 5, 6, 7, 9, 11, 13, 14, and 15. Ascocarps were developed when the mycelium from spore 2 was crossed with the mycelium from spores 1, 8, 10, and 12.

The observations presented in table 1 show that *Ascobolus geophilus* is heterothallic, since there are two sexual strains and no apothecia are produced unless both strains are present.

Five asci were used to determine the sexual strains of spores within a single ascus. The asci were designated A, B, C, D, and E. Table 2 and figure 1 give the results for ascus A.

TABLE 2.

			Spore number	
	1	2 :	3 4 5	6 7 8
1				+ + +
3	•	-	- -	+ + +
4 5	••			
6				
7	•	•••	•	

$$(4 \text{ and } 5) \times (4 \text{ and } 5) = (-)$$
  
 $(4 \text{ and } 5) \times 1 = (-)$ 

$$(4 \text{ and } 5) \times 8 = (+)$$

In ascus A, spores 1, 2, 3, 6, 7, and 8 were removed separately, while spores 4 and 5 could not be separated and were picked up together. After ger-

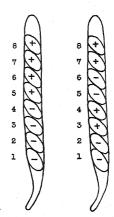


Fig. 1. Diagram illustrating segregation of sex factors in *Ascobolus geophilus* Seaver, ascus A (left), ascus B (right).

mination and growth, the mycelium from single spores was grown in all possible combinations. Spores 1, 2, and 3 proved to be of one strain, while spores 6, 7, and 8 were of the opposite strain. Spores 4 and 5 grown together failed to produce apothecia. This result was not expected from the arrangement of the other spores in the ascus and indicated that either the two spores were of the same strain or one had failed to germinate. Since three spores were of one strain and three of the opposite strain, it was obvious that spores 4 and 5 could not be of the same genetic constitution for sex. In order to determine which spore failed to germinate, the mycelium from spore 4 or 5, as yet undetermined, was grown with the mycelium from spore 1 and with the mycelium from spore 8. In combination with spore 1, no ascocarps were produced; in combination with spore 8, ascocarps developed. The evidence obtained indicated that spore 4 germinated, while spore 5 did not. Since in ascus A, as shown in table 2 and figure 1, spores 1, 2, 3, and 4 are of one strain and spores 5, 6, 7, and 8 of the opposite strain, sexual differentiation takes place at the first division of the zygote nucleus.

TABLE 3.

		Spore number													
-		1		2		3		4	- 1	5		6		7	8
	1	٠.	•									:			
	2					+									+
	3			٠				٠.				٠			
	4														
	5	٠.													
	6														
	7							•							
	g		•					•				٠.		•	• •

$$(6 \text{ and } 7) \times (6 \text{ and } 7) = (-)$$
  
 $(6 \text{ and } 7) \times 2 = (+)$ 

The results are shown for ascus B in table 3 and figure 1. Spores 2, 3, and 8 were removed separately, spores 6 and 7 remained attached to each other and

were removed from the ascus together, and spores 1, 4, and 5 failed to germinate on the agar. Mycelia from the three single spores were crossed in all possible combinations. Mycelia of spores 6 and 7, grown together, produced no apothecia, but when grown with the mycelium from spore 2, fruiting bodies developed, indicating that it was spore 7 rather than spore 6 which had germinated. Though all of the spores from this ascus were not available, those that germinated were in their proper order in the ascus. The results shown in table 3 and figure 1 indicate that spores 1, 2, 5, and 6 are of one strain, while spores 3, 4, 7, and 8 are of the opposite strain. It is evident that sex segregation in ascus B takes place at the second division of the zygote nucleus.

In ascus C, spores 2 and 3, 4 and 5, 6 and 7, were removed in pairs, while spore 8 was picked up separately. Spore 1 was lost. Each of the spore combinations, 2 and 3, 4 and 5, 6 and 7, produced apothecia, indicating that spores 1, 2, 5, and 6 are of one sex, while spores 3, 4, 7, and 8 are of the opposite sex. In ascus C, the segregation of sex factors is at the second division, as in ascus B.

In ascus D, spores 1, 2, 3, and 4 were removed together and allowed to germinate. Apothecia developed from this combination. Spores 5, 6, 7, and 8 were also picked up together. After germination, they, too, produced apothecia. The spores of ascus

E were removed in the same manner with similar results. This indicates that sex segregation in neither ascus took place at the first division of the zygote nucleus but occurred at the second or third divisions. Since segregation of the sexual factors in asci B and C is definitely shown to occur at the second division, it is presumed that such is the case in asci D and E. Therefore, the segregation of the factors for sex in the asci designated C, D, and E is as diagramed for ascus B in figure 1.

The results obtained with the five asci indicate that in Ascobolus geophilus sex factors are segregated at either the first or the second division of the

zygote nucleus.

### SUMMARY

Ascobolus geophilus Seaver is heterothallic, since the mycelium from a single spore will not develop apothecia unless crossed with the mycelium from a spore of the opposite strain.

Four spores from a single ascus are of one strain

while four are of the opposite strain.

Segregation of sexual strains takes place at either the first or the second division of the zygote nucleus.

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# THE TRACHEAL ELEMENTS IN THE FERNS 1

Mary C. Bliss

THE EARLIER botanists used the term "vessels" rather loosely as referring in general to the conducting elements of the vascular tissue, but it seems clear that in some cases at least the elements referred to as vessels were typical tracheids. Such for example are the "Treppengefässe" of Weiss (1878) illustrating the conducting elements of Lycopodium. The tracheal elements of the Ferns are referred to by most writers as tracheids, although Gwynne-Vaughan (1908) concludes that the tracheal elements in many ferns are vessels. The present investigation was undertaken to examine in detail the tracheal elements of representative types of the eusporangiate and the leptosporangiate ferns as classified by Eames (1936). These types include twenty-four genera of eight families as follows: Botrychium of the Ophioglossaceae, Angiopteris of the Marattiaceae, Osmunda and Todea of the Osmundaceae, Gleichenia of the Gleicheniaceae, Lygodium of the Schizaeaceae, Alsophila and Cyathea of the Cyatheaceae, Cibotium and Dicksonia of the Dicksoniaceae, and fourteen genera of the Polypodiaceaenamely, Adiantum, Asplenium, Blechnum, Davallia, Drynaria, Dryopteris (Nephrodium), Nephrolepis, Onoclea, Pellaea, Polypodium, Pteridium, Pteris, Thelypteris, and Woodwardia.

True vessels are characterized by specialization in the terminal portion of the element and this specialization is expressed in the development of definite, usually large, openings of various kinds and usually also by the angular orientation of the end walls with respect to the side walls. Interpreted in harmony with this definition of the vessel, true vessels exist in Pteridium latiusculum (Desv.) Maxon (Pteris aguilina L.). The first statement of this fact as far as I can determine was made by Russow in 1873. Sachs (1874), DeBary (1884), and Strasburger (1891) figure these vessels in several aspects, and the observations of these early anatomists have been confirmed by Jeffrey (1917). Figure 1 shows the vessel element in Pteridium latiusculum isolated from macerated material of the stem. The side wall facing the observer is marked by scalariform pits through which the pitting of the opposite side, irregular because of contact with parenchyma cells, is visible. The end walls are clearly at definite angles with the side walls. Figure 2 shows a higher magnification of the terminal portion of a vessel element in which the scalariform perforations of the end wall are evident. In an earlier paper (Bliss, 1921), the writer figured portions of these vessels in section showing clearly the difference between the side and end walls (fig. 3).

Typical vessels, in which the scalariform perforations of the end wall are also evident, are present in the root as well as in the stem of *P. latiusculum* (fig. 4, 5). In these figures the end wall is comparatively

<sup>1</sup> Received for publication June 6, 1939.

short, but in some cases the perforated area is very long. In one element, for instance (fig. 6), the writer counted 160 perforations; this indicates that the end wall must have met the side wall at a very small angle, and the area of contact must have been extensive.

Through the courtesy of Professor Jeffrey of Harvard University the writer was able to investigate another species of *Pteridium* which he had collected in Australia—namely, P. esculentum (Forst.) Nakai. In this species, which is closely related to P. latiusculum, vessels are also present in abundance. The elements vary in length, are clear cut in section, and are characterized in general by narrow scalariform bordered pits in the side wall and scalariform perforations in the end wall which is definitely at an angle with the side wall (fig. 7, 8). In a study of the material of both these species in root and stem, it is interesting to note that there are many cells that may be interpreted as transitional between the tracheid and the vessel element. In contrast to the typical vessel element where the end wall stands definitely at an angle with the side wall, there is often a cell that is a vessel element that tapers gradually to a point as does the tracheid and in which the area of contact between the contiguous elements in the terminal perforated region is much more extensive than it is in a typical vessel. This condition is evident in figure 9. There is every gradation from this very long end wall to the short end wall, oriented almost at right angles to the side wall and showing as few as six perforations. These conditions offer a possible explanation of the origin of the vessel from the tracheid in this genus.

Following the investigation of Pteridium, nine species of Pteris were studied. In none of these was there evidence of vessels such as have been described for the two species of Pteridium. The elements were in all cases extremely long, of medium width, and narrowly tapering at the ends. These observations on the length and width of the tracheal elements in the Ferns confirm those made in this group by Bailey and Tupper (1918) in their study of the comparative length and width of the tracheal elements of the vascular cryptograms, Gymnosperms, and Angiosperms. Figure 10 illustrates the type of tracheal element characteristic of the genus Pteris L. In most cases the lateral wall is marked by a single series of elongated slit-like, scalariform pits, uniform throughout the length of the element. There are undoubted instances of serial and irregular pitting in the lateral walls of the element as noted by Gwynne-Vaughan (1908), but in many cases, if traced far enough, the type of pitting is seen to be related to the fact that the element is overlain by parenchyma cells and is not here in contact with another tracheal element.

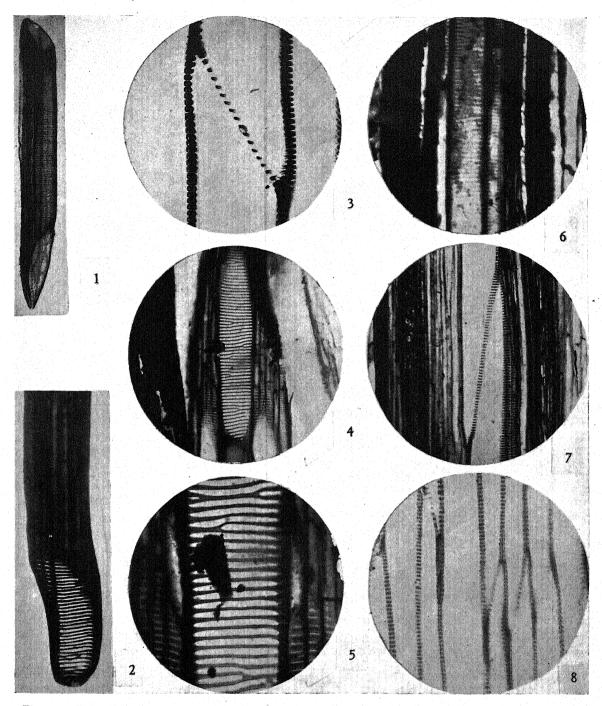


Fig. 1-8.—Fig. 1. Single vessel from macerated material of *Pteridium latiusculum*. ×40.—Fig. 2. Terminal portion of a vessel from macerated material of *P. latiusculum*. ×80.—Fig. 3. Portion of vessel of *P. latiusculum*, showing end wall in section. ×350.—Fig. 4. Longitudinal section of root of *P. latiusculum* showing end wall of vessel in face view. ×100.—Fig. 5. The same as fig. 4. ×350.—Fig. 6. Longitudinal section of root of *P. latiusculum* showing portion of end wall of vessel in face view. ×120.—Fig. 7-8. Longitudinal sections of stem of *P. esculentum* showing end wall of vessel in profile. ×150.

Following the investigation of the genus Pteris L., thirteen other genera of the Polypodiaceae were examined—namely, Adiantum (Tourn.) L., Asplenium L., Blechnum L., Davallia Sm., Doodia Br.,

Drynaria Bory, Dryopteris Adanson (Nephrodium Rich.), Nephrolepis Schott., Onoclea L., Pellaea Link., Polypodium (Tourn.) L., Thelypteris Schmidel, and Woodwardia Sm. In general, the tracheal

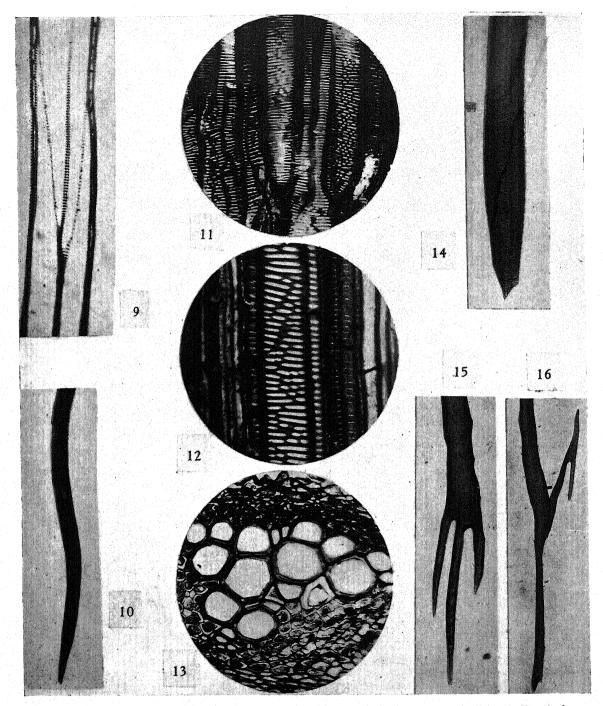


Fig. 9-16.—Fig. 9. Longitudinal section of stem of *P. esculentum* showing vessels. ×150.—Fig. 10. Terminal portion of a tracheid from macerated material of *Pteris quadriaurita*. ×60.—Fig. 11. Longitudinal section of stem of species of *Woodwardia*. ×125.—Fig. 12. Longitudinal section of petiole of species of *Dicksonia*. ×125.—Fig. 13. Transverse section of xylem of stem of *Osmunda cinnamo mea*. ×125.—Fig. 14. Terminal portion of a tracheid from macerated material of *O. regalis*. ×160.—Fig. 15-16. Terminal portions of branched nodal tracheids from macerated material of *O. regalis*. ×60.

elements are typical tracheids, very long, with sharply tapering ends. The lateral walls are usually marked by narrow scalariform pits. But in all of the genera investigated there was a tendency toward more or less irregular pitting on the lateral walls, the pits being small and circular or elliptical in outline. Various writers on the subject refer to Russow and to the fact that he found true vessels in the root of Nephrodium Filix-mas as in Pteris aquilina, because the end wall was perforated. These writers quote Russow incorrectly, as will be seen from the following excerpt from Russow's work. "Erwahenswerth ist das Vorkommen von wirklichen Gefässen in der Wurzel von Athyrium Filix-femina: die Enden der Gefässzellen sind leiterartig durchbrochen, ganz entsprechend der Perforation bei Pteris aquilina." (Christensen, 1906, gives Nephrodium Filix-femina as a synonym for Athryium Filixfemina.) This statement by Russow is the only one that the writer has found in the literature that asserts, as the result of original investigation, that there are vessels in Athyrium Filix-femina (L.) Roth (Nephrodium Michx.) similar to those found in "Pteris aquilina." In my study of macerated material and sections of the stem of two species of Nephrodium, N. denticulatum Fée, and N. mólle (Jacq.) R. Br., now respectively Dryopteris denticulata (Sw.) O. Ktze. and Dryopteris dentata (Forsk) C. Chr., there is no evidence of perforations in the end wall of the element. The xylem elements taper to a point, dove-tailing into one another, and the side walls are characterized by narrow scalariform pits.

Only one genus of the Polypodiaceae examined, Woodwardia Sm., showed in the tracheal elements any marked variation from those observed in Pteris L. Here the tracheids are characterized by comparatively blunt ends and are much shorter and wider than in most of the genera investigated. The lateral walls are especially conspicuous because of their marked irregular pitting. Figure 11 shows, at the right of the photograph, the terminal portion of one of these elements. Examples of this phenomenon could be multiplied, but the element figured will serve to illustrate a condition which is very common in the tracheal elements of this genus. It is an interesting fact that in the smaller branch veins, the narrower tracheids are characterized by scalariform pits, a condition which one would expect to find in the younger bundle.

In the genus Angiopteris Hoffm. of the Marattiaceae and in the genus Lygodium Sm. of the Schizaeaceae the tracheids are typically scalariform and taper gradually at the ends. In Cibotium Kaulf. and Cyathea Sm. of the Cyatheaceae and in Alsophila R. Br. and Dicksonia L'Her. of the Dicksoniaceae the tracheids are in general entirely scalariform but round or elliptical pits are not uncommon (fig. 12). There is no evidence of vessel structure.

The vascular elements of Gleichenia of the Gleicheniaceae are larger in diameter than those of many of the genera investigated and as a specialized vine type offered an interesting possibility of a more advanced condition in the tracheal elements, but here again there was no evidence of vessels. The tracheids are clearly scalariform and tapering at the ends.

I have left to the last the consideration of the two families, Ophioglossaceae and Osmundaceae, since these groups possess features in the vascular cylinder of the stem that are characteristic of the vascular cylinder of the seed plants. In the Ophioglossaceae as illustrated by Botrychium Sw., we have an advanced type of vascular cylinder: siphonostelic with collateral bundles, endarch xylem, and a small amount of secondary wood formed by the activity of a cambium. This type of central cylinder is not found in any living representatives of the true ferns and represents the mode of organization of the central cylinder characteristic of the stem of the seed plants. Although the topography of the cylinder is advanced in structure, I find no evidence of vessels. The tracheal elements are tracheids which resemble in a striking manner the tracheids of the Gymnosperms by the presence of fairly typical bordered pits. These observations confirm those of Wright (1920) that "the uniseriate and biseriate pits in Botrychium are large, round to oval in shape with a centrally placed pore.'

The woody cylinder in the Osmundaceae is also advanced in that the ectophloic siphonostele is made up of endarch collateral bundles but these are without secondary growth. Gwynne-Vaughan (1908) holds that the xylem of the Osmundaceae consists of true vessels, and he further states that the prevailing idea that the elements in question are tracheids is erroneous. Gwynne-Vaughan bases his conclusions on evidence derived from an investigation of Osmunda (Tourn.) L. and Todea Willd. He states that "there is no pit membrane closing the pits and separating the cavities of the contiguous tracheae from one another (the term 'tracheae' here refers to the elements generally interpreted as tracheids) and that the pits are in fact actual perforations." Conditions similar to those figured by Gwynne-Vaughan are seen in the transverse section of the xylem of Osmunda cinnamomea L. (fig. 13), where absence of the pit membrane is seemingly apparent in the slitlike openings in the walls of the tracheids. Sinnott (1910) also, in a similar section of the xylem of Todea hymenophylloides, shows the same condition of apparent absence of the pit membrane in the walls of some of the tracheids, while in others the pit membrane is clearly visible. The presence or absence of the "split" between the walls of the tracheids, according to Halft (1910), is due to the amount of cohesion between the bars of secondary thickening. Gwynne-Vaughan's paper is well known and need not be discussed in detail here, especially since later writers, Halft (1910), Bancroft (1911), and Wright (1920) have shown that Gwynne-Vaughan's conclusions are incorrect. By means of careful micro-chemical tests and physical experiments they have demonstrated that, with the exception of those types already mentioned, the pit membrane is present in the end wall and in the side wall of the xylem elements of the majority of ferns, including the Osmundaceae. In fossil types the absence of the pit membrane as noted by Kidston and Gwynne-Vaughan (1907, 1908) and Kershaw (1910) may, according to Seward (1898, 1910), be due to the action of ferments secreted by fungi or may be caused by the contraction and splitting of the membrane during drying or decay.

My investigation of Osmunda (Tourn.) L. and Todea Willd. shows that the tracheal elements under discussion are not markedly different from those found in the other genera. Serial pitting is not uncommon, but the elements are in shape characteristically tracheids with narrowly tapering ends (fig. 14). In the macerated material the dissolution of the walls of the tracheid into spiral bands several bars in width, described by Gwynne-Vaughan (1901) for Loxsoma R. Br., is clearly evident. The branched and nodal tracheids described by Boodle (1901) for Lygodium Sw. and Trichomanes Sw. are very numerous in Osmunda cinnamomea L. and O. regalis L. The most common form is the bifurcate type with the branches strongly divaricate, but often the branches are branched again, so that there is an interweaving of branch-tips which makes the separation of the individual elements difficult. Extreme examples of two of these branched tracheids are seen in figures 15 and 16.

#### SUMMARY

True vessels exist in Pteridium latiusculum (Desv.) Maxon and in P. esculentum (Forst.) Nakai. These vessels are characterized by narrow scalariform bordered pits in the side wall and scalariform perforations in the end wall.

The tracheal elements of all the ferns investigated, with this exception, are tracheids. The tracheids are in general scalariform, but a tendency to serial and irregular pitting on the lateral wall is not uncommon.

Since various investigators have shown that the pit membrane is present in the side wall and in the end wall of the xylem elements of the majority of ferns including the Osmundaceae, Gwynne-Vaughan's conclusion that these elements are true vessels is incorrect.

An interesting condition observed often in the root and stem of Pteridium is that of tracheal elements that taper to a sharp point as in a typical tracheid and that have an extensive and clearly perforated area of contact between two contiguous elements. Every gradation is found between this very long end wall and the very short end wall oriented almost at right angles to the side wall as in the typical vessel. This condition may be regarded as transitional between the tracheid and the vessel in this genus.

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# CHANGES IN INTERCELLULAR RELATIONSHIPS DURING THE GROWTH AND DIFFERENTIATION OF LIVING PLANT TISSUES <sup>1</sup>

Edmund W. Sinnott and Robert Bloch

The processes of growth and differentiation in multicellular plants involve changes not only in individual cells but in intercellular relationships. Young meristematic cells, all much alike in size and character, soon become differentiated into a wide variety of elements. Some remain small, others increase in volume even to several thousandfold. Some become elongated, others flattened, and others modified in various ways. How the changes which take place in one cell are related to those in neighboring cells is evidently an important problem, since it involves the more general question as to the developmental relations of cell and organism.

As to how the intercellular readjustments made necessary by differentiation are brought about there is no general agreement. Several possibilities suggest themselves. (1) Cells might slide along the surfaces of their neighbors and thus alter their positions. This commonly occurs in animal development, where cells may migrate for some distance. Ever since the work of Krabbe (1886) and other early students of the problem, a number of botanists have presented evidence that such "sliding growth" occurs in plant tissues, though this has been seriously questioned by others. (2) Some cells might cease division and begin expansion while their neighbors were still dividing, and thus become relatively large and make contacts with an increased number of cells. This method is certainly operative in many cases, though by no means in all. (3) Adjustments might be brought about by differential growth within cells, one portion of a cell exactly keeping pace with a rapidly growing neighbor cell and another portion with a more slowly growing cell, so that different parts of the same cell would thus grow at different rates. Such unequal or localized growth certainly takes place in root hairs, tyloses, and other tissues where one portion of the cell is free, but is more difficult to demonstrate in cells which are surrounded by others. (4) For a brief period when cells are very young and their walls may still be subject to surface forces, readjustments in length and relative position of the walls may take place which do not involve either sliding or differential growth in the strict sense of these terms.

It is often difficult to determine by a study of sections of killed material alone (the method heretofore chiefly employed) which of these possibilities of intercellular readjustment are actually operative. To obtain convincing evidence, it is obviously necessary to make successive observations of a group of cells in their living condition as they grow and differentiate. The purpose of the present paper is to bring

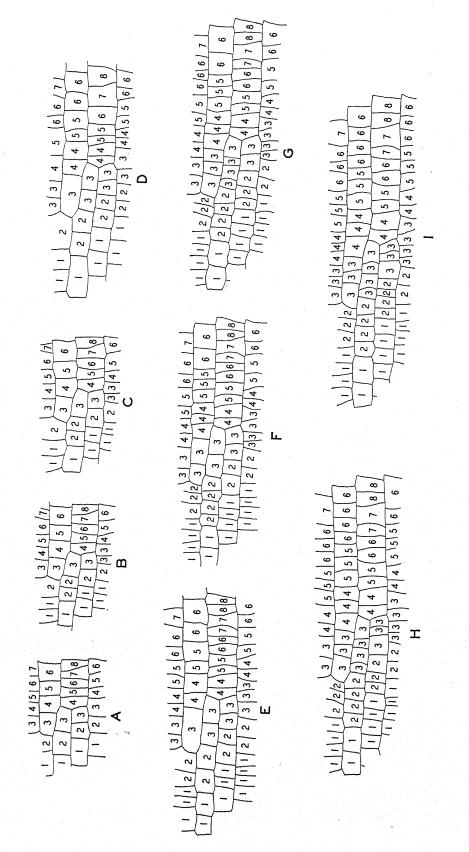
<sup>1</sup> Received for publication June 7, 1939.

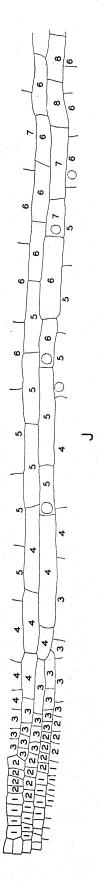
This investigation was carried on by the aid of a grant from the Special Fund for Research of Columbia University. forward such evidence from a study of the developing surface layer of the root in a number of species.

Methods.—One of the authors (Sinnott, 1939) has recently described a technique by which it is possible to study living plant meristems directly. Seeds of certain small-seeded grasses are grown on damp lens paper in moist chambers. The terminal root meristems are sufficiently translucent so that the cells of the outermost layer and the one directly beneath it can be observed under high magnifications. The members of a group of young meristematic cells, and of all their descendents, may be identified in this way from their earliest condition near the extreme tip of the root, through the period of cell division and cell enlargement until their final size is established and differentiation is complete, a process occupying from 24 to 48 hours. By successive camera lucida drawings made at intervals, a complete record may thus be obtained of every cell in an entire cell lineage, both as to its own changes and as to its relations with neighboring cells.

Roots of Phleum pratense, Poa trivialis, Sporobolus cryptandrus, and Chloris gayana were the ones chiefly studied. Aside from the fact that it can be observed in the living condition, such material has certain other advantages. The cells are rectangular and in regular longitudinal rows. Cell divisions in the surface layer (the tissue chiefly studied) are all anticlinal and with rare exceptions are at right angles to the long axis of the root. Cell enlargement is entirely in the longitudinal direction. A considerable degree of differentiation occurs, for the cells which are to develop root hairs are in many respects unlike those which do not do so. In Phleum and Poa this differentiation is more pronounced and occurs earlier than in Sporobolus and Chloris (Sinnott and Bloch, 1939). The exact points of origin of new cell walls, the increase in size of individual cells, and any alterations in the relationships between cells, can thus be studied under the simplest of conditions and can be expressed in quantitative terms.

RESULTS.—The basic results can be set forth most directly by presenting a series of drawings of the same group of cells at successive intervals from early development to maturity. This has been done for a group of surface cells in two roots of Phleum pratense in figures 1, A-J, and 2, A-D. In the first drawing in each root every cell is given a number, and since a region was always chosen in which one or more distinctively shaped cells were present, the original cells or their descendants can always be identified in later drawings. After division has ceased, all the cells elongate greatly, but they show no change in their transverse dimensions. It will be observed that numerous cell divisions occur and that following the last division the apical daughter cell ordinarily develops a root hair but that the basal one





does not. These presumptive root-hair cells grow less rapidly than do the hairless cells so that there is a considerable increase in diversity of cell size in mature tissues as compared with the meristem.

"Sliding Growth."—This increasing difference in relative cell size makes it possible to determine with some certainty whether or not the cells are sliding along one another. A study of the figures will make it clear that the position which a new cross wall assumes, with reference to the cross walls in the two adjacent rows, is essentially maintained throughout development and persists even when the cells are many times their original length. If sliding growth were to occur, the end wall of one cell should occasionally be seen to move past the end wall of its neighbor in the next adjacent row. This was never found to be the case, however, even though end walls in adjacent cells are sometimes laid down rather close to one another. Sliding growth would also be demonstrated if the distance between the end walls of two adjacent cells were ever seen to grow less, even though the change were not enough to carry the two walls past each other; but although distances between end walls of adjacent cells change markedly during development, this change always involves an increase rather than a decrease in the distance between them. In some cases of abnormal growth, as in the root treated with 0.02 per cent solution of colchicine (fig. 3), the cell walls grow very unevenly, and the distance between adjacent cross walls may fail to increase significantly even though the rest of the cell expands greatly; but it never decreases.

Throughout elongation, in this cell layer and characteristically for similar plant tissues in general where cells are in regular rows, each cross wall in one row tends to be opposite the middle of the adjacent cell in the next row and very rarely or never opposite another cross wall. In other words, the cells "break joints." If sliding growth took place, however, this characteristic behavior would not occur, for there should then be no relationship between the positions of the walls in one row and those in the

This same constancy of relationship is observable between cells of the surface layer and those immediately beneath it. Figure 4 is a drawing of a number of cells in these two layers near the meristem, and of the same cells during elongation and when fully expanded. Slight apparent changes in relationship will appear in such cases if the angle of observation is not exactly the same, but it is clear that the relationship of cross walls in the upper layer to those in the lower layer remains essentially constant.

Additional evidence that the wall of one cell does not slide along that of its neighbor is obtained from a study of the wall itself. In *Phleum* and *Poa* the presumptive root-hair cells (trichoblasts) are early differentiated from the hairless cells in a number of

respects. They are smaller, grow less rapidly, and are more richly protoplasmic. In the living condition their walls seem much like those of the hairless cells; but if the roots are treated with concentrated potash, the walls of the young root-hair cells tend to retain their firmness, whereas the longitudinal walls of two adjacent hairless cells become shortened and wrinkled (fig. 5A). It is significant that when a root-hair cell is in contact with a hairless cell, the longitudinal wall of the latter does not wrinkle, as it would do if free to slide along its neighboring cell, but retains its smoothness as it would do if tightly adherent to the much firmer wall of the root-hair cell (fig. 5B).

All the evidence, therefore, indicates that in this material, at least, the cells do not slide along one another but are held so firmly together that points in adjacent cell walls which are originally opposite each other are still opposite after these cells have

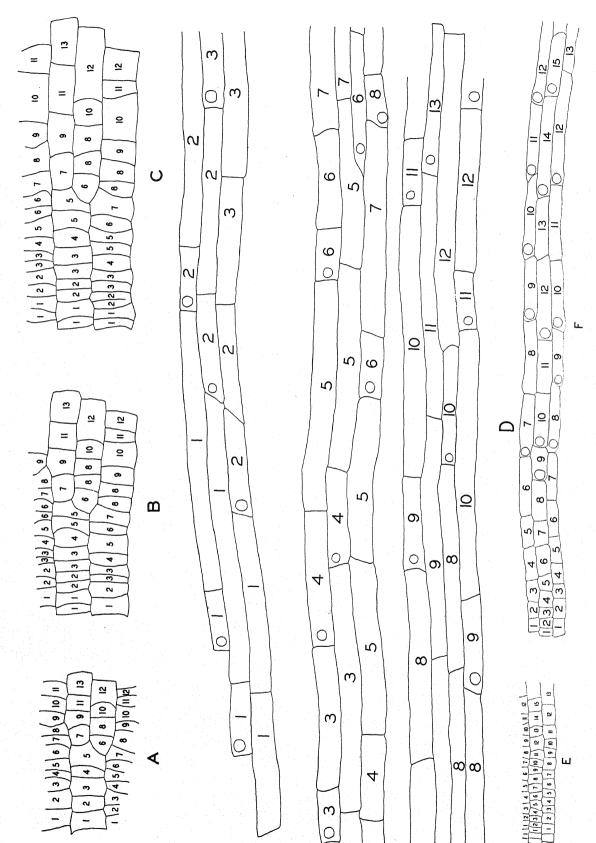
greatly elongated.

Differential wall growth.—If cells do not slide along each other, then the changes in intercellular relationships which arise after cell division has ceased must be due to differential cell growth. This will express itself primarily in differential growth of the cell walls. To determine whether this occurs and to compare the growth of various parts of a single cell, one must be able to identify specific points on the cell wall and to measure the distances between them at successive periods during growth. Since the walls of most thin-walled cells, such as the ones here studied, appear externally homogeneous, it seems difficult to find, such marker points within the cell itself. If adjacent cell walls are firmly fastened together, however, as they seem to be, and if they increase in length synchronously and without sliding, then the points along a given cell wall where the cross walls between its neighboring cells abut upon it may be used as constant measuring points. When the distances between such points are measured during growth, it becomes clear that the cell wall is expanding at different rates in different

This is most obvious, of course, in a comparison between the transverse and the longitudinal walls. The former do not grow in length after they are first laid down, but the latter may elongate thirtyfold or more. This marked difference occurs in any plant tissue like this where cell enlargement is primarily in one dimension rather than in three.

Elongation of the longitudinal walls, however, does not proceed at a uniform rate but may be shown to be more rapid at some points than at others. Various factors are evidently involved. First, if two cells are growing at unequal rates, then an adjacent cell which is in contact with both of them must evidently be growing faster opposite the faster growing cell than opposite the slower one. The root-hair cells grow somewhat less rapidly than the hairless cells,

Fig. 1. Phleum pratense. A group of surface cells at the meristem and their descendants, drawn at successive intervals during development. The root apex is toward the left in all figures. A, April 6, 10.50 a.m. B, 1.25 p.m. C, 4.45 p.m. D, April 7, 9 a.m. E, 11 a.m. F, 1 p.m. G, 2.50 p.m. H, 4.30 p.m. I, 6.40 p.m. I, April 8, 1.10 p.m.  $(A-I, \times 335; I, \times 205)$ .



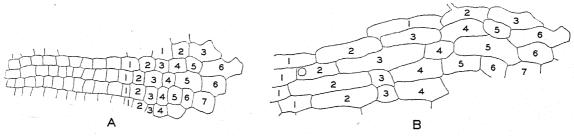


Fig. 3. Phleum pratense. Surface cells from a root treated with 0.02 per cent solution of colchicine, showing differential wall growth but persistence of original relative positions of cross walls. A, December 20, 11 a.m. B, December 21, 11 a.m. (×190).

and they evidently check the growth of cells adjacent to them. For example, cell 5, left, in the middle row of figure 2D is in contact with three cells in the row below it—5, 6, and 7. The first and third are hairless cells, but 6 is not. A comparison of figure 2D with B and C shows that the lower longitudinal wall of 5 has elongated much more rapidly at its ends, opposite cells 5 and 7, than in the middle, opposite 6. Similarly, the upper wall has elongated much more opposite cell 5, a hairless cell, than opposite cell 6, a root-hair cell. Similar relations can be found

(cf. fig. 2, C and D). This can be determined here because there is a marker relatively near one end of the cell. The upper wall of this same cell, marked in the middle, grows about equally on both sides of the marker, but this tells us nothing as to the growth near the ends as compared with the middle. The slower growth near the end walls is more pronounced in certain roots and under certain conditions. Thus in figure 2E, F, where size differences between cells are less pronounced, the growth of the cells is so much greater in the middle than at the ends that the

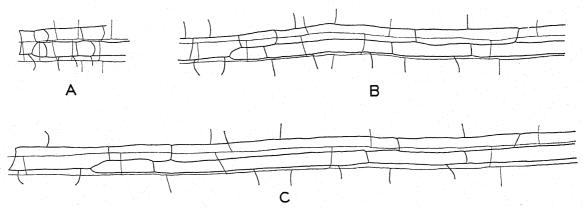


Fig. 4. Phleum pratense. Three stages showing the growth of the surface layer of cells (heavy line) and of the cell layer immediately beneath it (lighter line). The original relationships of the cross walls are essentially maintained during cell expansion. A, April 28, 12 m. B, 4 p.m. C, April 29, 10 a.m. (×255).

throughout. These differences in growth necessarily follow if cells are growing at different rates and if their walls do not slide past each other.

A second factor in differential wall elongation is the influence of the cross wall. In many cases, and especially under certain conditions, wall growth in length is much less in the vicinity of the end wall than in the middle of the cell. Thus in the middle row of figure 2D, cell 4 is cut by the wall between cells 5 left and 5 right below, and rather near its apical wall. This part of cell 4 (between the 3-4 wall, middle row, and the 5-5 wall below) evidently has grown much less rapidly than the rest of cell 4

cross walls in adjacent rows at maturity tend to fall almost in a row across the root, although when laid down they were each nearly opposite the middle of the adjacent cells. This condition seems to occur frequently in roots where growth is stopped or temporarily slowed down owing to age or repeated use for observation, or because of periodic changes in external conditions. In such cases considerable radial swelling of the growing region may take place, involving a certain amount of expansion of the cross walls.

A third factor in differential cell elongation is the gradient in expansion which progresses from the

Fig. 2. Phleum pratense. A-D, a group of surface cells and their descendants, drawn at successive intervals. A, June 3, 9.30 a.m. B, 1.50 p.m. C, 4.45 p.m. D, June 4, 9.50 a.m. E-F, two stages from another root, showing slower growth near the ends of the cells than at their centers. E, 10.30 a.m. F, 6.30 p.m.  $(A-C, \times 405; D, \times 265; E-F, \times 175)$ .

Table 1. Lengths of basal and apical portions, as percentages of total cell length, for five typical cells measured at six successive periods (fig. 6).

	May 16 9.30 a.m.	May 16 1.50 p.m.	May 16 4.45 p.m.	May 17 11.25 a.m.	May 17 2.25 p.m.	May 18 11.20 a.m.			
Basal, %		61 39	62.5 37.5	66 34	64 36	61 39			
Basal, %		46.5 53.5	$50.5 \\ 49.5$	50 50	47 53	47 53			
Basal, %		44.5 55.5	45.5 54.5	53.5 $46.5$	50 50	44 56			
Basal, %	. 62.5	68 32	68 32	76 24	74 26	68 32			
Basal, %	. 76	76 24	82 18	80 20	76 24	78 22			

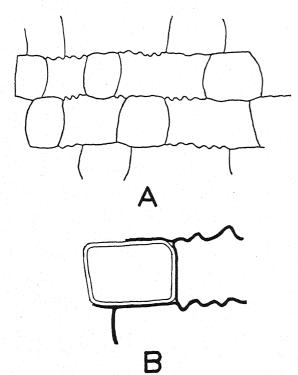
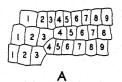


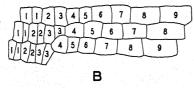
Fig. 5. Phleum pratense. Young root-hair cells and adjacent hairless cells after treatment with concentrated KOH, showing differences in the behavior of the wall in the two types of cells. A, after 10-minute treatment. B, after 30-minute treatment.

base of the root toward its tip. If a given group of cells in one row is carefully observed from the time

when cell division stops until elongation has ended, it will be seen that in each cell the basal (proximal) portion, as measured by marker points along it, grows more rapidly at first than the apical (distal) portion, and later that the apical portion grows faster than the basal. This same result is progressively visible in the successively more apical cells of the row. It is as though there were a "wave" of elongation rolling down the root, reaching its crest in one end of the cell first and then passing on to the other, the amplitude of the wave extending over the whole region of elongation. Since one cell occupies only a small part of this zone of extension, the differences between its two ends are not very conspicuous, but careful measurement reveals them.

This can best be studied in a form like Sporobolus where there is no marked differentiation between root-hair cells and hairless cells in early development. Figure 6A-C shows a group of cells, from a root of this genus, extending from the apex well into the region of elongation. In table 1 are given the percentage lengths of marked basal and apical ends of various cells in this figure, measured at six successive periods, only three of which are here figured. Measurements are made from one end of the cell to the point opposite a cross wall in the adjacent row. It is evident that at first the basal end gains faster than the apical and then the apical faster than the basal, the final proportion between the two being essentially like the original proportion. This may be seen, for example, in cells 5 and 6 of the middle row. The older cells, at the right in figure 6, have gone through both phases, and the original relations to their neighbors are restored. The much younger cells at the left are just entering the wave of enlarge-





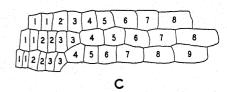


Fig. 6. Sporobolus cryptandrus. Three successive stages, showing differences in expansion between basal and apical ends of the cells. A, May 16, 9.30 a.m. B, May 17, 11.25 a.m. C, May 18, 11.20 a.m. ( $\times$ 390).

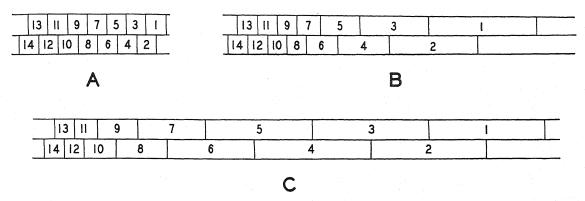


Fig. 7. Diagrammatic representation of the "wave" of elongation in a group of cells similar to those in fig. 6. A, cells of meristem before beginning of expansion. B, cells 1-7 have elongated more at their basal ends than at their apical ends. C, cells 1-5 have elongated more at their apical ends than at their basal (as compared with B), thus tending to restore the original proportions of the wall; cells 6-11 are now in the stage of greater basal growth.

ment, and their basal ends are growing more rapidly than the apical ones. There are slight irregularities in certain cells, probably due to errors in drawing, but the general behavior of the cells is as previously described. A diagram of three stages in elongation, with the changes considerably exaggerated, is shown in figure 7.

The same result is evident in the cells of *Phleum*, but here it is masked somewhat by the different growth rates of root-hair and hairless cells. In *Sporobolus*, also, when differentiation of these two types of cells begins to occur, just after the third stage here shown, the effect of the "wave" is much less obvious. It seems probable that the type of differential cell growth here described is characteristic of all similar tissues which are elongating by cell enlargement. If cells do not slide along each other, and if there is a gradient in root elongation, one end of a cell must evidently grow at a different rate from the other.

Discussion.—Of the four possible methods mentioned earlier in this paper by which changes in cellular relationships may be achieved, three seem inoperative in the tissue here studied. Differential duration of cell division does not occur; growth differences arise when the cell walls can no longer be modified by surface forces; and sliding growth seems to be eliminated. Differential cell growth is thus apparently the method by which intercellular adjustments, due to unequal cell enlargement, are brought about in this case.

Differential wall growth.—The problem in the present material, therefore, is concerned primarily with differential growth of the cell wall, since changes in the size and shape of a plant cell are chiefly related to changes in its wall. Most studies of the cell wall have been confined to single cells and have thrown little light on intercellular relationships. Since a cell is a relatively homogeneous physiological unit, it might be expected that its wall would grow equally throughout. This seems to be rather generally the case as far as wall thickness is concerned. In expansion, however, it is clear that

different parts of the cell may vary considerably. Thus a root hair grows in length only near its tip; and callus and tylosis cells, cambial initials, and certain latex cells show strongly localized growth. Our knowledge of the way in which the cell wall grows is as yet too meager for an understanding of how these local differences in growth are brought about, but that they occur even in unspecialized tissues like those here described seems obvious.

Some of these differences are caused by unequal growth of adjacent cell walls. The walls of the roothair cells, for example, grow less rapidly in length than do the walls of hairless cells. The decreased growth of a hairless cell at the point where it is in contact with a root-hair cell seems primarily due to the fact that the two cells are tightly bound together and that the slower growth of one checks the otherwise more rapid growth of the other. It appears that these differences in growth and differentiation between the walls of root-hair and hairless cells, which become noticeable from the time when both arise from the same mother cell, are associated with differences in the chemical and metabolic character of these cells, as demonstrated by microchemical tests made during the various stages of development. These changes are not always constant, as is shown by the various types of development here described, and they may easily be influenced by alterations in external conditions, such as the action of light or the calcium content or acidity of the culture solution. Normally in a type like Phleum the cells which are to form root hairs are distinguishable at the beginning from hairless cells by their somewhat more meristematic appearance, by their denser protoplasmic contents, and by staining more deeply with Ruthenium Red. As stated previously, their walls appear to be more rigid and tend to swell more with concentrated KOH (fig. 5, B). Both types of cells develop a birefringent cellulose wall which is surrounded by pectic material, but differences develop in the amounts of these materials laid down. The wall of the root-hair cell often tends to become thicker, and polar differences also gradually develop between its proximal end and the distal end, where the root hair arises.

The less rapid growth sometimes observed near the ends of a cell is perhaps to be explained as due to a change in character of the wall and to the amount of material laid down in the corners. In many plant cells the walls are somewhat thickened in this region. In the surface cells of the oat coleoptile recently pictured by Farr and Sisson (1939, fig. 1) it is noteworthy that the ends of the longitudinal walls seem much more rigid than the middle portions.

The differential wall growth within single cells due to the "wave" of elongation evidently results from still another factor. The lengthening of the tissue can best be pictured as due not to successive increase of one cell after the other but rather to the progressive elongation of the entire series of cell walls, sweeping up from the base toward the apex of the root and affecting all the walls at a given level equally, regardless of what cells these walls happen to form a part. The appearance suggests the passage of some stimulus from base to apex or the diffusion distally of some substance stimulating wall growth, independently of individual cells.

It seems clear, at all events, that one of the major factors in differentiation and in the changing intercellular relationships among plant cells during development is the unequal growth of various portions of the cell wall in the same cell. An understanding of how this is accomplished must wait until much more complete knowledge of cell wall development is obtained than is now available.

Other types of differential wall growth.—The material here described has the great advantage that it can be observed in the living condition. It is a comparatively simple tissue, however, with relatively little differentiation, and one would be unwise to draw from it too general conclusions as to the manner in which intercellular readjustments are brought about in other plant tissues. Various modifications

of this general type have been reported.

In many cells, for example, differences in wall growth are much more sharply localized than in the tissue discussed in the present paper. Thus in the crystal cells of *Citrus* described by von Guttenberg (1902) it is clear that during cell enlargement the upper portion of the wall, which ultimately reaches the surface of the leaf, grows very much more than does the lower portion, though both are increasing. A similar behavior of other crystal cells and of hair cells has been reported by Rothert (1900) and Knoll (1905).

Still more extreme is the differential growth of initials of prosenchyma in the secondary tissues of higher plants. Attention was first called by Sanio (1872) to the increase in length of xylem cells in successive annual rings. This increase has been shown by a number of later investigators to be due to an elongation of the cambium cell wall sharply localized at the ends of the cambium cells, the rest of the wall showing no elongation at all. Klinken

(1914) has studied this process by means of serial sections, and Neeff (1914) provides much evidence, especially from stems where cell polarity has been disturbed by decapitation. Bailey (1923) and Bailey and Kerr (1934) have also discussed this type of apically localized cambial cell growth. A somewhat similar type of growth is found in latex ducts of the non-articulate type. Zander (1928) and Schaffstein (1932) among others have shown that the growth of these structures takes place only near their tips, in regions where the other tissues of the plant are still growing.

These cases of sharply localized differential wall growth are thus confined to rather specialized meristematic tissues. They differ only in degree, however, from the simpler cases described in the pres-

ent paper.

Sliding growth.—Wall growth which is thus localized at a small region of a cell, however, or at the two ends of a long cell, necessarily brings about changes in intercellular relationships, since the elongating portion grows in between two neighboring cells and thus often radically changes the cell pattern. This is especially true of elongating cambium cells. It has been clearly demonstrated by Neeff (1914), who presents evidence that the sharp expanding cell tip digests the cementing substance between two neighboring cells, ruptures the protoplasmic connections, and grows between the two cells. The same change possibly occurs near the tips of latex ducts. This type of growth alters the relative positions of cells and is evidently an important factor in certain kinds of tissue differentiation. Unfortunately, it has been referred to by many workers as "sliding" growth, though there is no evidence that the surface of one cell slides over another during the process. The growth of the intruding cell may be thought of as that of a root hair moving through the soil by apical growth, or of a tylosislike structure which pushes out into a mass of tissue instead of into a cavity. The general picture of how such a change in intercellular relations could be brought about without sliding growth is shown in a rather simple case in the diagrams in figure 8, which is modified from von Guttenberg's data. The separation of the two epidermal cells a and b is brought about by the greater growth of the upper portion of the crystal cell c, though all the cell walls are growing somewhat. It will be noted that all points on the cell originally opposite each other remain so except those in the anticlinal wall between the epidermal cells a and b, but that these points, having established contacts with the newly developed portion of the wall of c, retain these connections without later sliding. To distinguish this type of changing cell relationship, the term intrusive growth is here pro-

Of course the possibility cannot be excluded that true sliding growth, where the surface of one cell actually slips over the surface of another, may sometimes take place. Krabbe (1886) in an extensive monograph, comes to the conclusion that sliding

growth occurs in very many plant tissues. He studied secondary xylem particularly, though most of his evidence concerns changes in the relation of vessel cells to adjacent smaller cells as the former increase in diameter. He finds that a vessel initial, originally a member of a regular radial row of cambial cells as seen in cross section, by its enlargement causes adjacent cells to be pulled apart from each other so that the young vessel cell, sliding over their surfaces, finally establishes contact with many more cells than it had at first.

Krabbe's results have not received substantial confirmation. Nordhausen (1897) showed that in many cases which Krabbe cited as sliding growth, the increase in number of cell contacts which a vessel gains as it increases in size is due to continuation of divisions in adjacent cells rather than to separation of cells. A similar explanation will doubtless hold for many other cases, notably the elongating vascular initials near the primary meristem, which ultimately are in contact with very many adjacent pith or cortex cells, the latter having continued to divide transversely for some time after the provascular cells ceased to do so.

Although the possibility of true sliding growth, in Krabbe's sense, cannot be excluded, it seems probable, in view of the evidence in the present paper and from the results of others, that it is rare or absent in most plant tissues and cannot be regarded as

an important factor in development.

Priestley (1930) has endeavored to explain changes in intercellular relationships by assuming the occurrence of what he terms "symplastic" growth, which presumably does not involve sliding. This conception seems to refer to adjustments in the cell wall brought about only at an early stage of cell growth, and therefore cannot be applied to the changes described in the present paper, which often continue throughout the development of the cell.

The issue of sliding growth involves more than a problem in histology, however, for it is connected with the fundamental problem of the relation of the cell to the organism in development. If cells slide along each other, the possibility is open that they may change their original positions and may actually migrate, suggesting the movements of the myxamoebae in the growth of the fructifications of certain myxomycetes. Such a changing relationship would tend to support the conception that the development of the organism is simply the result of complex interactions between the semi-autonomous cellular individuals of which it is composed. If, on the other hand, the cells are tightly adherent (though they may send out intrusive processes into adjacent tissue) the development of such a system could more readily be explained on the assumption of a mechanism inherent in the whole cell complex and not related to the behavior of independent units within it. At all events, any facts which will throw light on the nature of intercellular relationships, even of the relatively simple spatial sort here considered, cannot fail to have an important bearing on the enigma of organic development.

### SUMMARY

The problem of changing intercellular relationships brought about by growth and differentiation was studied in living tissues in the growing region of certain grass roots.

Cellular readjustments observed in this material cannot be due to surface forces, since they appear relatively late, after cellulose walls have been laid

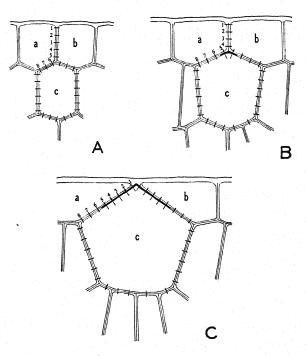


Fig. 8. Diagrammatic representation of the mode of growth of the crystal cells of the leaf of *Citrus* (modified from von Guttenberg), showing the intrusive type of cell growth. See text.

down; nor due to differential cell division, since division ceases in all the cells of a given region at about the same time. They apparently are not due to "sliding" growth, since transverse walls in adjacent longitudinal rows do not approach or pass each other.

Changes in intercellular relationships here observed result from differing rates of growth in different parts of the cell wall. This is evident where a cell is in contact with two others of unequal growth rates; where the ends of a cell grow less rapidly than the middle portion; and where the "wave" of elongation, proceeding toward the root apex, affects one end of a cell before it does the other.

Other more specialized cases of differential walls growth are briefly discussed, such as the more or less sharply localized intrusive growth of certain idioblasts, cambium cells, and non-articulate latex ducts.

Reported instances of sliding growth are reviewed, and it is shown that they may be explained by differential growth of the cell wall.

The implications of these results for the general problem of development are discussed, and the im-

portance of a more extensive knowledge of the structure and growth of the cell wall is emphasized.

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## A NEW TIBOUCHINA FROM PERU 1

## H. A. Gleason

Tibouchina incarum Gleason, sp. nov. Frutex ramosus, caulibus strigosis; foliis tenuibus ovato-lanceolatis acuminatis basi cuneatis utrinque pubescentibus 7-pli-nerviis; panicula ramosa glanduloso-hirsuta; hypanthio poculiformi glanduloso-hirsuto; staminibus fere isomorphis, connectivis basi breviter productis; T. Gayanae affinis.

Widely branched shrub to 15 dm. high; stems 4-angled, closely strigose. Petioles slender, substrigose, 1-2 cm. long. Leaf-blades thin, ovate-lanceolate, to 11 cm. long and 4.5 cm. wide, acuminate, minutely serrulate, abruptly cuneate at the base, closely substrigose above, paler and softly villous on the veins and veinlets beneath, 7-pli-nerved. Panicle ample, freely branched, to 25 cm. long, its branches hirsute with chiefly glandular hairs. Pedicels about 2 mm. long. Flowers 6-merous. Hypanthium cupshaped, thin-walled, 4-4.5 mm. long, densely glandular-hirsute. Sepals triangular, often somewhat falcate, recurved, 2.6 mm. long, finely ciliate, sepa-

<sup>1</sup> Received for publication August 1, 1939.

rated by narrow sinuses. Petals bright pink, broadly obovate, 8 mm. long and almost as wide. Stamens nearly isomorphic but differing slightly in size. Filaments 4.7 or 3.7 mm. long. Thecae stoutly subulate, 4.3 or 3.8 mm. long. Connectives prolonged 0.7 or 0.8 mm. Ovary superior, densely bristly at the summit. Style 10 mm. long; stigma truncate.

Machu Picchu, near Cuzco, Peru: in shaded moist hedgerows, alt. 5,000 ft., Balls B 6817 (Type in the Britton Herbarium, New York Botanical Garden); grassy hillside, alt. 8,000 ft., Stafford 772 (Herb. Kew.); grassy slope among shrubs, alt. 8,000 ft., Stafford 791 (Herb. Kew.); open brush formation, alt. 2,200-2,300 m., West 6456 (Gray Herb.).

The species is closely related to *T. Gayana* (Naud.) Cogn., also from the vicinity of Cuzco, in which the leaves are smaller and rounded to subcordate at the base, the sepals proportionately longer, and the pubescence of the hypanthium not glandular.

THE NEW YORK BOTANICAL GARDEN

# CELLULOSE AS A SUBSTRATUM FOR SAPROPHYTIC CHYTRIDS 1

# R. H. Haskins

SINCE APRIL, 1938, collections of saprophytic chytrids have been made from bait placed in ponds near the Thames River on the campus and in a sphagnum bog at North Bay, Ontario. The method of baiting was that used by Karling (1935).

A number of these chytrids are being kept in culture. In general, methods and technique followed are those described recently by Couch (1939) and brought here by Miss Berdan from his Chapel Hill laboratory after the summer of 1938. All cultures have been kept under as nearly sterile conditions as possible in a room with controlled temperature (about 60°F.). Until January 30, 1939, the substrata used were leaves of wheat, corn, rye, oats, other cereal grains, and grasses from which the chloro-

phyll had been removed.

There are some practical disadvantages in their use. One is the necessity of keeping the plants in young stages all year in the greenhouse. Secondly, the thickness of the leaves, the presence of stomata, the epidermal hairs, and even the cell walls often tend to obscure the early stages of growth from the germinating zoospores. Very young, thin leaves are used to offset this, but their tissues break down rapidly, and the cultures require constant care. Even with older leaves, some chytrid species develop so rapidly that water and substratum have to be changed every few days. When several hundred cultures are being kept over long periods, the labor and inconvenience are excessive.

During a conversation in January, 1939, Dr. F. K. Sparrow, Jr., suggested the use of paraffined filter paper as a substratum, following Dr. Rex Webster (Middlebury College, Vermont), who had grown a species of Rhizidiomyces2 upon it. More than fifty years ago, according to Schröter (1889), Rhizophlyctis rosea (de Bary and Woronin) Fischer was found on moist blotting paper by Sorauer in Ger-

OBSERVATIONS.—It had been noticed in our laboratory that most of our forms seemed to thrive equally well in leaves lacking nearly all cell contents as in those (e.g., young wheat) with globules of brown oil. Consequently, lens paper (Cenco) in which the individual fibers could be distinguished under the microscope was substituted for paraffined filter paper. On January 30 a small rectangle of this was added to a healthy culture of Rhizophlyctis

<sup>1</sup> Received for publication June 13, 1939.

The writer wishes to express his thanks to Prof. H. B. Berdan, under whose supervision this work has been done, for advice and criticism, for revision of the manuscript, for reports on chytrids (6) to (10) and use of figures 4, 6, and 10; also to Dr. F. K. Sparrow, Jr., of the University of Michigan, and to Dr. J. S. Karling of Columbia University for their interest and advice.

<sup>2</sup> Since writing this paper it has been learned that Dr. J. N. Couch (1939) grew four species of chytrids on boiled filter paper in 1938, one of these being Rhizophlyctis rosea

and another Rhizophlyctis n. sp.

Petersenii Sparrow (?) which had been collected in London, Ontario, and grown in grass since April, 1938. On February 13, numerous orange sporangia typical of this chytrid were found in the meshes of the paper (fig. 12). The fibers seemed to be partly decomposed, and the rhizoids of the fungus twined loosely among them and even extended out freely

into the surrounding water.

That same day a small rectangle of cellophane (National Paper Goods, Ltd., Hamilton, Ontario, 1937 grade) was added to the culture dish. On February 15, innumerable zoospores in varying stages of germination (fig. 1) could be seen on both upper and lower surfaces of the nearly transparent cellophane by simply changing the focus of the microscope. In from two to three days' time, the thalli seemed to be penetrating into the cellophane by haustoria-like rhizoids (fig. 2). The old zoospore cases were often still attached to the germ tubes, and the fundamental swellings (the incipient zoosporangia) were plainly visible. The young thalli seemed at this stage to be extramatrical except for the tips of the "haustoria" which were apparently dissolving away the cellophane by some enzymatic (?) action comparable to that by which cell walls of leaves are punctured by invading rhizoidal tips. Later the thalli looked to be wholly or partially imbedded in cavities hollowed out of the cellophane (fig. 3). That plenty of nourishment was being obtained was obvious since the thalli duly matured (fig. 13), and the sporangia reached proportions larger than previously recorded (Sparrow, 1937), often measuring over 350 \( \mu \) and having from one to ten exit tubes. When mature, they produced vast numbers of zoospores, and reinfection of fresh lens paper, cellophane, and leaves was readily accomplished. As the cultures on lens paper and cellophane became very old, the substrata were reduced to jelly-like, amorphous masses containing the sporangia. At this stage single sporangia can be teased out easily under the binocular microscope to set up unisporangiate cul-

It was considered advisable to extend the use of these substrata to other chytrids in culture at the time. In all cases, conditions were kept as nearly sterile as possible. The rectangles of lens paper were autoclaved in a dry, stoppered flask. Cellophane was always boiled thoroughly in distilled water just before using. Results are summarized as follows:

Rhizophlyctis Petersenii Sparrow (?).—In some of the gross cultures pronounced, irregular zonations were observed in the cellophane as the rhizoids extended out farther and farther from the original central body of the thallus (fig. 7). In other cases the cellophane around the sporangia was stained a bright rose-pink color for a considerable distance. Sterile blotting paper was similarly stained by the chytrid. Ashless filter paper was tried to insure

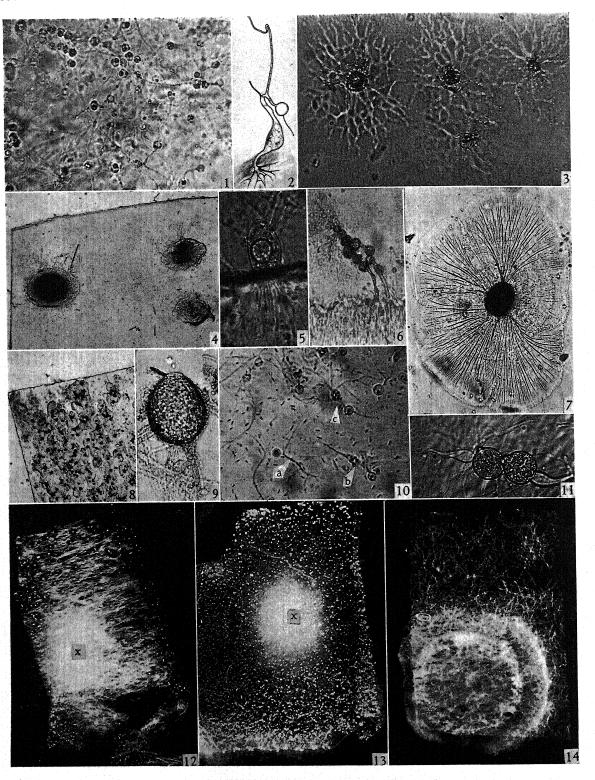


Fig. 1-14.—Fig. 1. Rhizophlyctis Petersenii Sparrow (?). Germination of zoospores on cellophane. From three-day culture. Note empty zoospore cases and sporangial fundaments. ×414.—Fig. 2. R. Petersenii Sparrow (?). Young thallus with "haustoria" just penetrating the cellophane. Note the old spore case, germination tube (of which there may be several) and swelling (sporangial fundament). Camera lucida drawing. ×690.—Fig. 3. R. Petersenii Sparrow (?). Young thalli in cellophane with rhizoids extending through the cellophane. ×414.—Fig. 4. No. 7. "Colonies"

against this stain being the result of certain impurities in the substratum. In some cases the whole rectangle became a bright orange-pink color due to the masses of orange sporangia plus this surrounding rose stain.

A single spore culture was made by the dilution method, using a capillary tube. Instead of blowing the final spore on to the substratum, it was left in a drop of sterile charcoal water on a sterile slide, and a tiny piece of sterile lens paper was added to this drop. The slide was placed in a sterile moist chamber. In three days' time the paper was examined, and since a single mature thallus showed, the paper was placed in a regular culture dish containing fresh substratum in charcoal water.

From this culture, growth was secured in all the materials mentioned above. All stages of development were seen, including resting spores (fig. 5) and their germination. A paper describing the morphology and development of this chytrid is now in progress. Since our fungus does not comply exactly with Sparrow's (1937) description of R. Petersenii and has not as yet infected insect exuviae, kindly sent us by him, it may prove to be a separate species or possibly R. rosea (de Bary and Woronin) Fischer.

Nowakowskiella elegans (Now.) Schroeter.— This chytrid has been grown in great profusion on both lens paper and cellophane since February 13, 1939. The rhizomycelium often extends out into the water all around the rectangle of substratum, giving a distinctly fuzzy appearance. Figure 11 shows two intercalary sporangia growing in tandem in the water over the upper surface of a piece of cellophane in which the rhizomycelium is growing. In one case on cellophane, the threads grew out to a distance of more than 5 mm. A few of these were pulled off with delicate sterile forceps and placed on a piece of sterilized lens paper (1.5 imes 1.0 cm.) in water. The threads invaded the paper radially from the point of inoculation, giving an appearance remarkably like the growth of higher fungi on agar (fig. 14). Subcultures made from this have been carried since April 1 without any other fungus ever being present. No resting spores were observed.

Nowakowskiella sp.—This species, collected in North Bay, is larger than N. elegans, and the sporangial wall has a brownish color. Single spore cultures were made on February 13 by the method described above. Growth was excellent on both lens paper and cellophane. No resting spores were found.

Diplophlyctis sp.—This form is larger than D. intestina (Schenk) Schroeter which we have grown in Nitella sp. Dr. J. S. Karling, who kindly examined our material, considers it to be a separate species from D. intestina which he has studied intensively (Karling, 1928, 1930, 1936a). Single spore cultures were made on February 16 as before. All stages of growth, including resting spores and their germination, were obtained on both the new substrata. Figure 8 shows numerous mature sporangia on cellophane. The cavities in which they lie are plainly visible.

CO.3—This is an operculate, monocentric form with very large sporangia (fig. 9). The spores are released in a vesicle. Cultures from single spores made as in (1) on February 13 gave abundant growth on both substrata. While it is still being carried in unifungal culture, no resting spores have been found to date.

Septochytrium variabile Berdan.—A strain was established by Miss Berdan (1939) from a single spore germinated on agar in August, 1938. In February of this year the new substrata were introduced by her into cultures of this chytrid. In lens paper the chytrid shows extreme polycentricity. In cellophane this character was somewhat reduced, but when it was shown, the threads of rhizomycelium with secondary sporangia were radially arranged upon the original central body or primary zoosporangium. A cellophane culture, begun on April 11, contained numerous resting spores which were germinating on May 9 as previously described by her.

Catenochytridium carolinianum Berdan (1939).

—This chytrid was the most difficult to grow on the new substrata. It is of the "endo-exogenous" type (Karling, 1936b), and the germ tube penetrates directly into the tissue from the zoospore. Several attempts were made before growth was accomplished on lens paper. On cellophane the spores usually flattened out, turned brown, and died without any penetration being accomplished. However, on May 5 a splendid culture developed, the sporangia producing spores which infected new cellophane. Figure 6

<sup>3</sup> New or unidentified chytrids in our culture room are temporarily designated by letters (Haskins) and numbers (Berdan).

in cellophane. ×14 (approx.).—Fig. 5. Extramatrical resting spore of Rhizophlyctis Petersenii Sparrow (?). Note the intramatrical rhizoids below and others extending outward from the spore. ×775.—Fig. 6. Catenochytridium carolinianum Berdan. Thallus extramatrical to cellophane except for tips of rhizoids. ×112.—Fig. 7. A single young thallus of Rhizophlyctis Petersenii Sparrow (?) showing zonation of rhizoids in cellophane. ×112.—Fig. 8. Diplophlyctis sp. growing in single spore culture on cellophane. ×14 (approx.).—Fig. 9. Empty operculate sporangium of "CO" growing on lens paper. Note the intermingled rhizoids and fibers of the paper. ×112.—Fig. 10. No. 7. Small portion of a square centimeter of cellophane covered by germinating zoospores in early stages of development. a, production of branching germ tube from zoospore; b, swelling (sporangial fundament) on germ tube; c, mycelium beginning to develop. ×414.—Fig. 11. Nowakowskiella elegans (Now.) Schroeter growing extramatrically from cellophane. ×414.—Fig. 12. Rhizophlyctis Petersenii Sparrow (?) growing in lens paper. Each white dot is a single zoosporangium. Note the reduction of the fibers to jelly-like mass at right. Round white area (X) due to light reflection while photographing. ×7 (approx.).—Fig. 13. R. Petersenii Sparrow (?) in cellophane. The white dots are individual sporangia. Note rhizoids extend into the water from the edges. Round white area (X) due to light reflection while photographing. ×7 (approx.).—Fig. 14. Nowakowskiella elegans (Now.) Schroeter in lens paper. Note the extension of the rhizomycelium into water. ×4 (approx.).

shows a young thallus, on which the sporangium has not yet developed, apparently growing in water outside the cellophane and with only the tips of the rhizoids imbedded in the cellophane. Later the sporangium developed and shed its spores. No resting spores have been observed yet.

No. 5.3—This is an inoperculate member of the Cladochytriaceae collected by Miss Berdan in Chapel Hill, June, 1938. The hyaline resting spores are subtended by a linear row of delicate cells somewhat similar to those described by Butler (1907) for Nowakowskiella ramosa. It grew well on both substrata, forming zoosporangia and resting spores both in the cellophane and on the rhizomycelium extend-

ing out far into the water from it.

No. 7.3—This is another inoperculate member of the Cladochytriaceae, with lemon-shaped to clubshaped sporangia, collected on the University campus by the writer and cultured since October, 1938, by Miss Berdan. It grows unusually well on both substrata, forms resting spores, and appears pale yellow in mass on cellophane. Figure 10 gives an indication of the clarity with which early stages in development can be seen in this form even when bacteria have not been washed off the cellophane. A few threads bearing zoosporangia and growing far out into the water were separated out and placed on the surface of a rectangle of cellophane. Figure 4 shows three separate "colonies" of young germinating zoospores apparently formed when three of these sporangia shed their spore balls which settled en masse upon the cellophane directly below them.

Cladochytrium replicatum Karling.—Lens paper and cellophane were placed in gross cultures containing this chytrid. It grew readily on both. Resting spores were formed.

Discussion.—Up to date, these substrata which consist mainly of cellulose have been used chiefly for the sake of convenience. They are available anywhere and in all seasons or can be transported simply when cultures have to be moved to a new laboratory as often happens during summer session. They are easily sterilized. Lens paper is particularly useful in establishing single spore cultures since the spore tends to be caught in the meshes of the paper. Cellophane is an excellent medium for the observation of early growth stages and for determining whether the tips of rhizoids are blunt or pointed, often a diagnostic feature. Once the chytrid has penetrated into it, bacteria are washed off its slipperv surface much more thoroughly than from other substrata we have used, while rotifers and protozoans develop sparingly in these cultures. Even when successive generations have been supported in it from one to two months' time the water in the culture dish remains fairly clean, and new cellophane may be added during this time without changing it. Also, the rate of growth generally seems slower in these substrata than in the leaves. Consequently, we have greatly reduced the labor involved previous to their introduction. Unfortunately, Pythium, Fusarium, etc., grow on them, so they have no particular value in freeing a chytrid culture from contamination of this type. Also, older thalli which are imbedded in cellophane are not always as easily studied as in leaves.

We are attempting to obtain a similar cellulose product in a form which can be reduced to the consistency of 2 per cent agar and autoclaved. So far all efforts have been unsuccessful. The common solvents are deadly to the delicate chytrid spores. We have been in touch with the research department of the Eastman Kodak Company, the DuPont Company. and the "Cellophane" Division of Canadian Industries. Limited, in the hope of securing viscose, the fluid intermediate product in the manufacture of "Cellophane" cellulose film. It is the sodium salt of cellulose xanthogenic acid dispersed in a dilute solution of caustic soda. Other dispersing agents for cellulose suggested by them are cuprammonium solution, solutions of zinc chloride, and certain of the tertiary amines, none of which has seemed to be practicable in connection with our problem.

It is not possible at this point to define exactly the relationship of the chytrid to these media. The distinction between intramatrical and extramatrical portions of the thallus is not so clearly established as on most other substrata. In lens paper, thalli have been seen to develop in the narrow lumen of a fiber, forcing the walls apart, in which case they would probably be called intramatrical. In other cases the same chytrid develops chiefly outside with only a few rhizoidal branches penetrating into the fibers. There seems often to be more extensive extramatrical growth as the substratum becomes filled with rhizoids of the chytrid and begins to break down. Is this due to the fact that the nutrient material has been used up or that it has become reduced into a substance soluble in the nearby water and consequently used there as food by the fungus? On the other hand, the very early stages (fig. 1, 10) seem to be almost completely extramatrical.

Karling (1936b) describes a case where a zoospore of Chytridium lagenaria Schenk germinated in the water surrounding the host cell to form a rather extensive thallus. He states that when branches of the rhizoidal system come into contact with the host wall and penetrate into the lumen of the cell, such thalli occasionally mature and produce zoospores. This has been a common occurrence during our experiments with these new substrata. Figure 6 shows this particularly well. Here only the tips of the rhizoids are imbedded in the cellophane. In figure 10, the rhizoidal tips appear to be seen through the cellophane, while the young thalli are in the water above it.

The majority of chytrids grow in pollen grains, algae, water molds, apples, dead wood, and roots and leaves of higher plants. The principal substance common to these is plant cellulose. From our evidence it seems reasonable to assume that, for a certain group of saprophytic chytrids at least, certain

commercial cellulose products may be substituted for these natural substrata.

### SUMMARY

Lens paper and cellophane have been used since January, 1939, as substrata for saprophytic chytrids.

Ten species of chytrids have been grown on these substrata. All have produced mature thalli and zoospores which reinfected fresh material.

Six species have produced resting spores. Germination of these resting spores has been seen in three species.

Culture of chytrids on the cellulose substrata reduces labor, keeps cultures cleaner, and shows young stages of development more clearly than other substrata used in our laboratory up to the present.

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# SEXUAL HORMONES IN ACHLYA. I. INDICATIVE EVIDENCE FOR A HORMONAL COORDINATING MECHANISM <sup>1</sup>

# John R. Raper

A NEW HETEROTHALLIC ACHLYA.—Since the discovery of heterothallism in the Mucorales by Blakeslee (1904), a number of fungi in the Zygomycetes and other groups have been shown to have a similar type of sexual relationship. Of these fungi a few members of the order Saprolegniales, belonging to the widely distributed water molds, have been reported: Dictyuchus monosporus Couch (1926), Sapromyces Reinschii Jordan (cited in Weston, 1938), Achlya bisexualis Coker (1927), and A. regularis Coker and Leitner (1938). In the course of the present work on hormonal coordination of the sexual process in these so-called heterothallic forms<sup>2</sup> the author has collected ten isolates which comprise a

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Contribution from the Laboratories of Cryptogamic Botany and the Farlow Herbarium, Harvard University,

The writer wishes to express his sincere thanks to Professor Wm. H. Weston, Jr., under whose guidance this investigation has been carried out, for his stimulating advice and constructive criticism. The writer is also most appreciative of the criticism and many helpful suggestions which he has received from Professor K. V. Thimann and Dr. D. H. Linder, and he is indebted to Mr. R. M. Page for the drawings reproduced here.

<sup>2</sup> A paper planned for publication in the near future will point out a number of constant and characteristic differences between the sexual condition in these partially separate-sexed forms and heterothallism as originally defined.

new species-complex belonging to the genus Achlya. As a large portion of the work reported here has been carried out with isolates of this new species, a description of the species is at first necessary.

Achlya ambisexualis sp. nov. (fig. 1-6).—Mycelium extensive; maximum diameter in water on hemp seed, 4.5-5 cm.; thin and loose. Hyphae slender, 20-65 μ, mostly 40- $50 \mu$  in diameter at base, tapering only slightly to tip. Gemmae abundant, formed by segmentation of vegetative hyphae, usually with diameter equaling that of parent hyphae; rarely branched; intercalary gemmae long, cylindrical,  $30-50 \times 250-2000 \mu$  in size; terminal ones usually tapering to a point; sometimes but infrequently breaking apart to float free in the water. Zoosporangia abundant, usually solitary; long clavate,  $25-40 \times 200-500 \mu$ ; greatest diameter being nearer terminal than basal end. Zoospores 8.5-10  $\mu$ , mostly 9.2-9.6  $\mu$  in diameter, emerging as usual for the genus, encysting at the tip of the sporangium to form a very loose hollow sphere. Oogonia and antheridia formed when sexually compatible strains are brought together. Sexual character of certain thalli variable, reacting as oogonial (2) or antheridial (3) thalli when crossed with strong ♂ or ♀ mycelia, respectively.3 Oogo-

3 This capacity for sexual reversal is a significant feature of the sexuality of this as well as other "heterothallic" watermolds previously described. The term gynandromixis or gynandromictic sexuality is suggested, implying the mixed sexual character of the different sexual strains and the capacity of certain strains to react as 3 and/or 9.

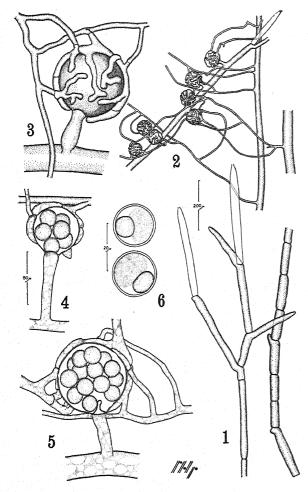


Fig. 1-6.—Achlya ambisexualis. The drawings in fig. 1-15 were made of living material in water with the aid of a camera lucida at initial magnifications of 220, 915, and 2100. For convenience absolute scales are included.—Fig. 1. Gemmae and emptied zoosporangia. ×55.—Fig. 2. Female hypha (left) with oogonia and β hypha (right) with antheridial hyphae—from a mating of β and φ mycelia. ×55.—Fig. 3. Immature oogonium and antheridia. ×225.—Fig. 4-5. Ooogonia in optical section showing oospheres. ×225.—Fig. 6. Mature oospores. ×525.

nia borne rarely intercalary, usually terminal on short lateral branches, 0.25-0.5 times diameter of oogonium, 0.5-4 times as long as diameter of oogonium; spherical in shape, occasionally oblong;  $50-100 \mu$  in diameter, 55 per cent within size 65-85  $\mu$ ; wall thin, less than 1  $\mu$ , smooth with broad pits under antheridia; approximately 50 per cent of oogonia having a blunt, thick-walled projection protruding into the oogonium from the tip of the stalk. Oospores 1-20 in each oogonium, rarely less than 3, mostly 8-14; 20-27  $\mu$ , 64 per cent, 22-24  $\mu$ ; eccentric; usually completely filling the oogonium. Antheridial hyphae much branched, 9-12  $\mu$  in diameter. Antheridia 9-10  $\mu$  in diameter; cylindrical, finger-like, and clearly cut off from antheridial hyphae; much branched, usually almost entirely covering the surface of the oogonium. Always one antheridium and usually many on each oogonium before oogonial delimitation and differentiation of contents to form oospheres can occur.

Seven isolations, all from Charles River at Cambridge, Massachusetts.

Achlya ambisexualis var. abjointa var. nov. (fig. 7-10). -Mycelium compact and dense; maximum diameter 2.3-3 cm. on hemp seed in water. Hyphae 35-70  $\mu$  in diameter at base and of approximately equal diameter throughout except for sharply tapering tip. Zoosporangia 30-50 ×350-600  $\mu$ ; clavate, broadest near terminal end. Spores 10-12  $\mu$ , discharged as normal for the genus, forming a loose hollow sphere at mouth of sporangium. Gemmae exceedingly abundant, formed by segmentation of vegetative hyphae; often branched; commonly breaking apart to float free in the water; terminal gemmae long and sharply tapering.  $330-530 \times 30-65 \mu$  at basal end, tapering to 15-33  $\mu$  near tip; intercalary gemmae much shorter, 110-160  $\times$  30-65  $\mu$ . Oogonia formed sparingly when compatible strains are mated; oogonia terminal on main hyphae or short lateral branches; spherical, rarely oblong; 50-85 µ in diameter, 75 per cent between  $55 \mu$  and  $70 \mu$ ; many with projecting

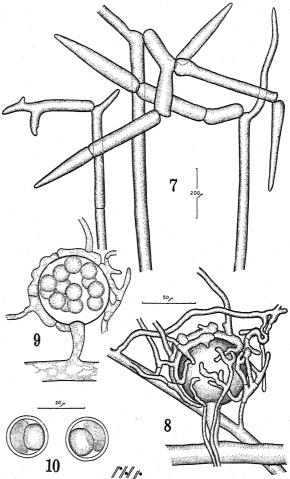


Fig. 7-10. Achlya ambisexualis var. abjointa.—Fig. 7. Gemmae. Note characteristic manner of breaking apart and occasional branching.  $\times 55$ .—Fig. 8. Immature oogonium and antheridia.  $\times 225$ .—Fig. 9. Oogonium in optical section to show oospheres.  $\times 225$ .—Fig. 10. Mature oospores.  $\times 525$ .

stalk. Oogonial wall thin and smooth with broad pits under antheridia. Eggs eccentric, 7–20 in each oogonium, 9–13 most common; 17–23  $\mu$  in diameter with 80 per cent between 18–20  $\mu$ . Antheridial branches highly branched and slender. Antheridia finger-like and much branched usually completely covering oogonial wall with their ramifications. Certain plants capable of behaving as either  $\delta$  or  $\mathfrak P$ .

Two collections: Barton Mills, England; Chapel Hill, North Carolina.

Achlya ambisexualis var. gracilis var. nov. (fig. 11-15). —Mycelium loose, diameter up to 3 cm. on hemp seed. Hyphae small, 20-40  $\mu$  in diameter at the base and tapering only slightly to the tip. Zoosporangia plentiful, long clavate, dehiscing as usual for the genus, with the encysted zoospores forming a very loose sphere at the mouth. Zoospores, 10-11  $\mu$ . Gemmae very abundant, formed by segmentation of the hyphae with no noticeable increase in diameter, frequently more than 1 mm. in length, but usually much shorter; not branched and rarely disjointing. Oogonia spherical, thin-walled;  $35-85~\mu$  in diameter, 57 per cent between  $50-60~\mu$ . Oospores eccentric, 3-15 per oogonium, usually 6-10, completely filling the oogonium; 18-

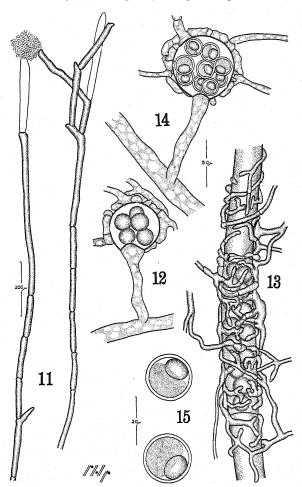


Fig. 11-15. Achlya ambisexualis var. gracilis.—Fig. 11. Gemmae and zoosporangia.  $\times 55$ .—Fig. 12-14. Oogonia and antheridia. Oogonia frequently in intercalary position.  $\times 225$ .—Fig. 15. Mature oospores.  $\times 525$ . (Fig. 12-15 from a mating of A. ambisexualis var. gracilis  $Q \times A$ . ambisexualis A.)

 $27~\mu$  in diameter, with 64 per cent between  $22-24~\mu$ . Antheridial branches and antheridia as in type variety.

Single collection: Blue Hills, Massachusetts.

Achlya ambisexualis sp. nov.—Coloniis in aqua grandibus et effusis, usque 4.5–5.0 cm. latitudine, hyphis 20–65  $\mu$  crassis. Gemmis abundantibus, per partitio hypharum formatis; zoosporangiis et zoosporiis generis propriis. Thallis gynandromictibis. Oogoniis globosis; 50–100  $\mu$ , 58 per cent e 70–80  $\mu$  diametro. Oosporiis 2–20 in oogonio quoque; 20–27  $\mu$  64 per cent e 22–24  $\mu$  diametro; membrana tenui; guttulis oleosis lateraliter positis. Antheridiis gracilibus, multis in oogoniis applicatis.

Achlya ambisexualis var. abjointa var. nov.—A typo differt coloniis angustioribus et laxioribus usque 3.0 cm. diametro, hyphis 35–75  $\mu$  crassis. Gemmis abundantibus, saepe scindentibus. Oogoniis 50–85  $\mu$ , 70 per cent e 55–70  $\mu$  diametro. Oosporiis 3–15 in oogonio quoque, 17–23  $\mu$ , 89 per cent e 18–20  $\mu$  diametro, membrana crassiore.

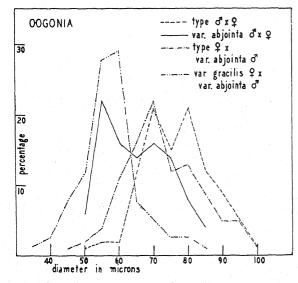
Achlya ambisexualis var. gracilis var. nov.—A typo differt coloniis angustioribus et densioribus, usque 3.0 cm. diametro; hyphis 20–40  $\mu$  crassis. Gemmis gracilibus et longis. Oogoniis 35–85  $\mu$ , 57 per cent e 50–60  $\mu$  diametro. Oosporiis 18–27  $\mu$ , 64 per cent e 21–23  $\mu$  diametro, membrana crassa.

Remarks on the taxonomy of A. ambisexualis.—Achlya ambisexualis belongs to the same section of the genus as A. klebsiana Pieters and the more recently described A. regularis Coker and Leitner. This new species has certain striking characters in common with both A. klebsiana and A. regularis. For example, all these plants are characterized by the formation of gemmae through the segmentation of the vegetative hyphae with very little or no increase in the diameter before or after their formation. A. ambisexualis may be separated from these species, however, by the size of the oogonia and oospores, by minor differences in the morphology of the sexual organs, and by morphological characters of the vegetative mycelium.

In so far as vegetative characters are concerned, A. ambisexualis var. gracilis agrees nicely with the description of A. regularis Coker and Leitner. However, the larger size of the oogonia and oospores, the wider range in size of these organs, and the absence of intercalary oogonia in A. regularis, prohibits the inclusion of this variety under that species.

A plant vegetatively very similar to certain of the isolates of A. ambisexualis was reported from Australia by Crooks (1937). This plant has gemmae characteristic of this species, but it produces oogonial initials which are never differentiated to form oogonia and oospheres. Crooks suggested that her plant was possibly a female strain of a heterothallic species of which the compatible  $\delta$  was lacking and, on the basis of incomplete knowledge of the sexual organs, designated it as Achlya sp. To the writer it seems that this plant is the same as that here described as A. ambisexualis var. abjointa.

Within the complex which is here given specific status, it is immediately obvious that three different varieties are involved. That they are closely related is shown by the fact that the sexual organs of all three varieties are identical except for size. An analysis of the distribution curves for the oogonia



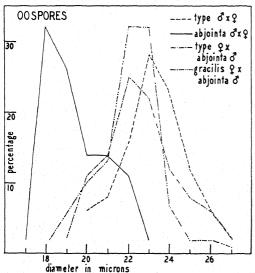


Fig. 16. Distribution curves of oogonia and oospheres of A. ambisexualis and its varieties, abjointa and gracilis.

and oospores of the three varieties clearly brings out these size differences (fig. 16). The type ? mated with the type & produces oogonia falling between  $50-100 \mu$ , with 58 per cent in the range  $70-80 \mu$ , and oospores from 20-27  $\mu$  with 64 per cent within the limits of 22-24  $\mu$ . Matings of var. abjointa type  $\mathfrak{P}$ with type & give sexual organs of the following dimensions: oogonia 50-85  $\mu$ , with 70 per cent, 55-70  $\mu$ ; and oospores 17-23  $\mu$ , with 80 per cent, 18-20 μ. A mating between a \$\pa\$ strain of the type variety and a & of var. abjointa produces sexual structures whose measurements are consistently slightly smaller than those produced in a mating of ô and ♀ plants of the type variety. The curves in the two matings are similar with more than 50 per cent overlap in both oogonia and oospore size. This is to be expected, since the same 9 plant was

used in both crosses, and it is from the  $\mathfrak P$  that both oogonia and oospores arise. A striking difference is seen when the isolate of var. *gracilis* is involved as the  $\mathfrak P$  of the mating pair. The size of the oogonia is nearer that of var. *abjointa*, 35–85  $\mu$ , with 57 per cent between 50–60  $\mu$ , while the size of the oospores more nearly agrees with that of the type variety, 18–27  $\mu$ , with 64 per cent, 21–23  $\mu$ .

Further indication of the close relationship of the three varieties is the fact that they are perfectly compatible in inter-varietal crosses. It is then chiefly a matter of immediate convenience that they are all included here in the same species; further collections of the three varieties and additional experimental work on the sexual relationships will be necessary before they can be given a more final and definite status.

The reason for the specific name ambisexualis is the dual sexual behavior of many of the isolates of this species. Of the ten plants collected, six have been shown to be capable of behaving as either  $\delta$  or  $\mathfrak P$ , depending on the stronger sexual potentialities of the mate.

Introduction and historical background.—In the course of an investigation of the sexuality in the species just described, it became increasingly obvious that initiation and coordination of the different phases of the sexual process were accomplished by diffusible substances. It seemed fitting, therefore, to investigate this phenomenon.

The first demonstration of coordination of the reproductive processes by diffusible substances in the fungi was given by Burgeff (1924) for a member of the Mucorales. In matings of (-) and (+) strains of Mucor mucedo he found that ordinary vegetative growth of the two mycelia stopped shortly before actual contact was established. Into this region, the "restraint area," only a few hyphae penetrated, and certain of these hyphae from each mycelium developed swellings and coralloid branches near their tips to form progametangia even before contact. He considered this characteristic swelling and branching as the initial stage in the sexual reaction and suspected that this effect was brought about by specific chemical substances diffusing between the two mycelia. His classical experiment of growing the two sexually compatible strains on opposite sides of a permeable collodion membrane proved this to be the case, for each of the paired mycelia was sexually activated. This experiment demonstrated that a diffusible substance (or substances) capable of passing through the membrane was responsible for the sexual activation of the plants. The reaction at a distance, induced by diffusible chemical substances, Burgeff termed "telemorphosis." Further, in a number of cases he saw the curvature of the sexual organ initials toward similar organs of the opposed strain, and this he termed "zygotropism."

The work of Burgeff offered the necessary stimulus for extensive investigations in this and other members of the Mucorales. Of the papers describing these investigations many added little or nothing to

Burgeffs results; a few, however, contain significant contributions to the knowledge of the sexual proc-

esses in the phycomycetous fungi.

Verkaik (1930), also working with Mucor mucedo, repeated the membrane experiment but found that only the (+) strain produced sexual organ initials under the experimental conditions. The evidence obtained indicated that only a single substance was involved, originating in the (-) strain and bringing about the formation of zygophores in the (+) strain. This work appears to have been done without adequate controls, and since other investigators have been unable to duplicate her results, its value is questionable.

Ronsdorf (1931), working with *Phycomyces Blakesleeanus* and using the membrane technique, found evidence for two mutually complementary substances interacting between the two mycelia to bring about telemorphotic responses in the two opposed strains. Attemps to extract these substances from the (—) and (+) mycelia were unsuccessful. An interesting point brought out in this work is the effect of histamine, which, when added to the medium, markedly increased the intensity of the sexual reaction. He suggested the possibility of close chemical relationship between this compound and the specific substances involved in the reaction.

Kohler (1935), using Mucor mucedo and the membrane technique, also obtained zygophore formation on the mycelia of both strains. He was unable to determine the origin of the reaction and was uncertain of the number of substances involved.

Krafczyk (1935), in experimental work on Pilobolus crystallinus, considerably advanced the knowledge of the sexual reaction in the heterothallic Mucorales, particularly as regards the hormonal activating and coordinating mechanism. Pronounced telemorphosis and zygotropism were repeatedly observed, and evidence was presented which indicated that the sexual reaction was under hormonal control until the time of gametangial delimitation.

For the aquatic Phycomycetes, the first evidence suggestive of a similar hormonal coordinating mechanism was given by Couch (1926). In working out the sexuality of Dictyuchus monosporus he observed the formation of oogonial initials at some distance from the nearest ô hyphae, other portions of the ? mycelium being in contact with that of the 3. The antheridial hyphae which finally reached the oogonial initials frequently originated from ô hyphae as far as 2 mm. distant, and the antheridial hyphae grew directly toward the 2 organ. Further evidence for diffusible sexual substances was seen in intergeneric matings with Thraustotheca primoachlya. From his observations Couch suspected a hormonal coordinating system and performed a number of experiments designed to reveal the presence of diffusible substances. A modification of Burgeff's membrane experiment was first used with the ô and ? plants separated by a thin collodion membrane; & and P plants were also grown close together in water, but not in actual contact. In both cases the

results were negative, neither of the mycelia giving any reaction.

Working with Sapromyces Reinschii of the Leptomitaceae, Bishop (1937) found definite evidence for diffusible sexual substances. He figured & and ? plants, growing on a semi-solid substrate, with large numbers of oogonia and antheridial hyphae on the and ô mycelia, respectively, in a region where actual contact had not yet occurred. He was further able, by introducing into a culture of the ô plant the water in which a \( \rightarrow \) had grown, to show a considerable increase in the branching of the tips of the ô hyphae; these ramifications he interpreted as antheridial branches. Approximately 3 per cent of the & hyphae showed this response as against 1 per cent in a control culture. The number of substances involved in the reaction, the loci of their production, and their specific actions were not determined.

The mode of growth of the aquatic Phycomycetes is strikingly different on semi-solid media from that of the terrestrial Zygomycetes. In the latter, with the exception of a few forms such as Pilobolus, the growth is almost entirely aerial, with only a limited inter-matrical mycelium. In the case of the aquatic Saprolegniales on the contrary, growth is almost entirely below the surface of the substrate. This characteristic manner of growth renders these submerged forms more amenable than members of the Mucorales to experimentation involving diffusible substances. In the latter the parts of the mycelium which give a sexual reaction and from which the specific substances would presumably arise, are normally above the surface and surrounded only by a film of water. In the case of Pilobolus, which grows submerged in the substrate and is not subject to this generalization, the ease with which diffusion experiments can be made compares favorably with similar experiments for those forms which are characteristically aquatic.

Because of the incompleteness of results and the limited scope of previous work on the coordinating mechanism in the sexual reaction of the aquatic Phycomycetes, the need for a more extensive investigation was apparent. For the purpose of such a study, Achlya bisexualis and A. ambisexualis were beautifully suited. Their manner of growth, the ease and simplicity of technique with which they can be cultured and employed in experimentation, and particularly the strong sexual affinities of certain of the isolates of the latter species made them the ideal experimental plants. The objectives of the investigation have been (1) the demonstration of hormones operative in initiating and coordinating the different stages in the sexual process and (2) the determination of the number of specific substances involved, the points of their origin, and the specific reactions which they induce.

The present paper presents those lines of evidence which indicate a hormonal coordinating mechanism in the sexual reaction of *Achlya*, and the probable mechanism is described. A second paper, planned for the near future will give an account of work con-

clusively proving the existence and activity of diffusible substances.

THE SEXUAL REACTION.—In order to present a comprehensible account of hormonal activity in Achlya, it is at first necessary to describe the re-

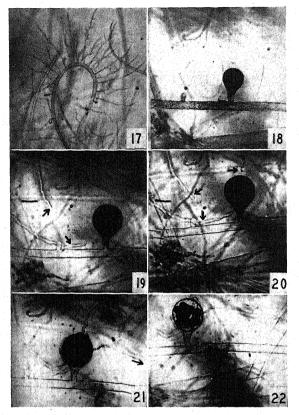


Fig. 17-22. Stages in sexual reaction between  $\beta$  and Q mycelia of Achlya ambisexualis. The photomicrographs here reproduced in fig. 17-27 were made of living material in agar.—Fig. 17. First stage, formation of antheridial branches on  $\beta$  hyphae along line of intermingling of  $\beta$  and Q mycelia.  $\times 30$ .—Fig. 18. Second stage, formation of oogonial initial; two were initiated but only one matured.  $\times 60$ .—Fig. 19-20. Third stage, directional growth of antheridial branches (indicated by arrows) toward the oogonial initial.  $\times 60$ .—Fig. 21. Fourth and fifth stages, delimitation of antheridia (obscure at this magnification) and oogonium.  $\times 60$ .—Fig. 22. Later stage in sexual reaction; the contents of the oogonium have been differentiated to form a number of oospheres toward which grow fertilization tubes from the antheridia.  $\times 60$ .

productive processes in some detail. To illustrate the different stages in the sexual reaction, the development of a single  $\circ$  organ and the accompanying  $\circ$  organs will be traced.

When ô and \$\varphi\$ plants are placed together, the first indication of a sexual reaction is the formation of numerous slender, gnarled, and highly ramified lateral branches on the vegetative hyphae of the ô. These characteristic lateral branches are the antheridial hyphae. They are apparently produced at

random on the  $\delta$  hyphae, and at this stage they certainly show no directional growth toward the P mycelium (fig. 17).

The second stage in the reproductive process is the formation of oogonial initials, first seen as short club-shaped lateral branches on the hyphae of the 2 mycelium. Such a branch elongates slightly, accumulates protoplasm, and begins to swell terminally to form a globose structure. The oogonial initial whose development is followed in figures 18 to 22 was observed two hours before the first photomicrograph was taken, and at that time two small lateral branches of similar size were seen located on the same hypha in proximity. During the two-hour period which followed, the branch to the right (fig. 18) grew and increased in diameter from 20 μ to 50 μ, which represents an increase in volume of approximately twenty times. This increase in volume and an increase in amount of protoplasm continues during a period of 4-12 hours, depending on the environmental conditions. Once the oogonial initial has reached its maximum size of 50-90 μ in diameter, no change is observed during a period of 12-24 hours, subsequent events depending on the accomplishment or failure of the second and third & reactions which follow.

The third stage of the sexual process is initiated when the antheridial hyphae, which at first ramify at random, begin to grow toward the oogonial initials. Figure 19, taken 16 hours after the initiation of the 9 organ, shows two antheridial hyphae approaching the initial. At the end of 17 hours, one of these slender filaments had reached the 9 initial and a third was seen growing toward it (fig. 20). At the end of 21 hours all three of the antheridial branches had reached their objective; upon touching the wall of the oogonial initial they grew in such a way as to become wrapped around that organ. In this particular case the antheridial hyphae originated from ô hyphae at distances of 600  $\mu$ , 530  $\mu$ , and 500  $\mu$  from the oogonial initial, and the path of growth in each case practically coincided with the shortest distances between the points of origin and the ? organ.

The fourth stage in the sexual reaction again concerns the antheridial hyphae. Beginning shortly after they become applied to the oogonial wall, protoplasm accumulates in their tips, and transverse walls delimit the  $\delta$  gametangia or antheridia from the parent antheridial hyphae. This process was completed 21 hours after the oogonial initial was first seen.

The fifth stage in the sexual process follows closely upon the completion of antheridial delimitation. A transverse wall is formed which delimits the large, densely filled portion of the oogonial initial from the stalk and the parent  $\,^\circ$  hypha (fig. 21). The spherical structure thus formed is the oogonium or  $\,^\circ$  gametangium. The formation of the basal wall in the oogonium occurs 24 hours after the initiation of the  $\,^\circ$  organ.

Later stages, including reorganization of the oogonial content to form oospheres, the inward

growth of fertilization tubes from the antheridia (fig. 22), and fertilization, follow, in that order, the delimitation of the oogonium.

While the above is a typical example of the sexual reaction, considerable variations may be seen if a number of cases are followed. The oogonium whose development was just outlined was approached by three antheridial hyphae. Others may attract a much larger number, as many as 86 having been seen growing toward a single \$\pa\$ initial. In such a case the oogonial initial and the numerous antheridial branches approaching it comprise a sphere with the former as its center and the latter forming radii in all planes. A large number of these antheridial hyphae may become applied to the \$\pa\$ initial so that in many cases the entire surface of the oogonium is covered by antheridia.

It often happens, when numerous oogonial initials are produced within a small area, or when the sexual reaction is interrupted, as described below, that antheridial hyphae do not reach the oogonial initials during the time they are receptive. In this case the oogonial initial may be renewed by proliferation. Lateral branches grow out from the bulbous portion of the initial and enlarge at their tips just as did the original. This process may be continued through a number of generations of initials so that from the original a complex of as many as 12 may eventually be formed. During this process there is no apparent increase in the amount of protoplasm, and, as a consequence, most of the initials are supplied with so little protoplasm that they are incapable of developing to maturity as oogonia. In some cases, however, where the process is not carried to such extremes two or sometimes three daughter initials may be functional in a perfectly normal manner after having been reached by the necessary antheridial hyphae.

EVIDENCE INDICATIVE OF A HORMONAL COORDINATING MECHANISM.—That the different stages outlined above are initiated and coordinated by a hormonal mechanism is indicated by the following experimental findings: (1) The sequence of events and the time intervals separating the various phases of the reaction indicate a hormonal mechanism. (2) Incompatibility in inter-specific matings of A. ambisexualis and A. bisexualis involves disruptions of the coordinating mechanism which seem to indicate not only the presence, but also specificity of sexual substances. (3) Variations in the composition of the medium frequently lead to changes in the sexual process which may be interpreted as break-downs in the coordinating mechanism.

In addition to these lines of evidence which point to a series of diffusible specific sexual substances initiating and coordinating the sexual process, telemorphotic reactions occur in both 3 and 9 plants. The results obtained from carefully manipulated experiments, making use of these distance reactions, constitute proof of the presence and activity of sexual hormones. These results will be embodied in a second paper of this series soon to appear.

SEQUENCE AND TIME INTERVALS OF STAGES IN THE SEXUAL REACTION.—A hormonal coordinating mechanism is strongly indicated by the sequence of events in the sexual reaction and the time intervals at which they occur. Characteristic differences in the time intervals of the events in agar and water matings necessitate the consideration of each in some detail.

(a) Sequence and time intervals—in agar matings.—The course of developments in the sexual reaction can easily be followed when compatible & and P plants are mated on a suitable agar medium in Petri dishes. The medium best suited for this purpose was found to be one containing 0.3 per cent soluble starch, 0.1 per cent peptone, 1.0 per cent lentil (hot water extract), 2.0 per cent agar, and trace quantities of KH2PO4, MgSO4, CaCl2, FeCl<sub>3</sub>, and ZnSO<sub>4</sub>. Each plate was inoculated with the two sexually opposed strains, the inocula being placed at some distance so that the two resulting mycelia would occupy the greater part of the plate before contact occurred. This allowed for a long line of intermingling of the hyphae of the two plants. By inverting the plates and examining them with a dissecting or compound microscope, the matings could be observed without exposure to air-borne contaminants.

When compatible & and & plants are mated on lentil-extract agar, the first indication of a sexual reaction is the formation of antheridial branches near the tips of the & hyphae (stage one described above). For a period of 6-10 hours the only change to be seen is an increase in the number and length of these 3 branches, but after this period the 2 hyphae located nearest the activated portion of the & mycelium begin to produce oogonial initials at their tips or on short lateral branches (stage two). The time interval between the appearance of the antheridial branches and the oogonial initials indicates the diffusion of a substance from the & mycelium to the  $\mathcal{P}$ , initiating the production of oogonial initials. That oogonial initials are formed only after production of antheridial hyphae suggests the probability that such a substance is produced by the antheridial branches.

Beginning at the time the oogonial initial reaches its maximum size, 6–8 hours after its initiation, antheridial hyphae begin to grow directly toward it (stage three), and this directional growth continues for as long a period as 10–12 hours. After this period the oogonial initial exerts no influence over the growth of the antheridial hyphae, growth again occurring at random or toward other oogonial initials which are in the receptive stage. These facts indicate that a specific substance is produced by the oogonial initial for a period of approximately 12 hours after it reaches maximal size and that this substance is responsible for directional growth of the antheridial hyphae.

Once the tips of the antheridial hyphae have become applied to the wall of the organial initial, the delimitation of antheridia takes place (stage four), and it is only after a period of 2-4 hours after this

formation of the basal wall in the antheridium that the oogonium is likewise delimited from its stalk and parent ? hypha (stage five). The constancy of this time lapse is significant, and suggests that some substance is produced by the antheridium, after its delimitation, which is necessary before the oogonial initial is capable of becoming transformed into an oogonium.

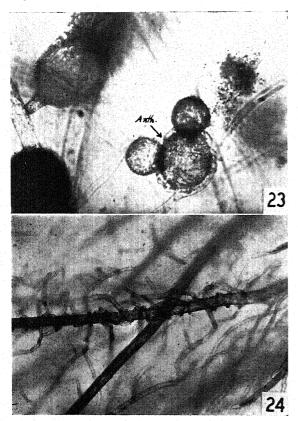


Fig. 23–24. Inter-specific matings of Achlya ambisexualis with A. bisexualis.—Fig. 23. Sexual reaction in inter-specific matings of Achlya ambisexualis  $Q \times A$ . bisexualis A. Numerous antheridial hyphae are applied to each oogonial initial (as shown at anth.) but neither antheridia nor oogonia are delimited.  $\times 100$ .—Fig. 24. A. ambisexualis  $A \times A$ . bisexualis  $A \times A$ . Disexualis  $A \times A$ . Disexualis  $A \times A$ . Disexualis  $A \times A$ . These are attracted to and become coiled around the vegetative hyphae of the  $A \times A$ . Usually no further reaction in either thallus.  $A \times A$ .

(b) Sequence and time intervals—in water matings.—The same sequence of events and the same proportionate time intervals are seen in water matings. These matings are made by bringing together vegetatively mature mycelia, growing on hemp seed, as described by Raper (1936).

In water matings of the same plants used for the preceding observations, the time intervals may be considerably shorter, antheridial branches appearing within two hours of the time that the pairings were made. This interval occurring between the time

that the two plants are placed in juxtaposition and the time of the appearance of the antheridial hyphae indicates that for the formation of the  $\delta$  initials some substance produced by the vegetative P plant is necessary. Thus the activity of the P in initiating the entire sexual process is suggested.

Beginning about 2 hours after the appearance of antheridial branches, oogonial initials start to appear along the line of intermingling of the two opposed mycelia. These enlarge rapidly and are soon producing the substance which attracts the antheridial branches. Finally, antheridial delimitation and oogonial delimitation occur in sequence soon after the ô initials reach the Q organ. In a mating of strongly compatible plants this entire process may be accomplished as soon as 8 hours after the plants are placed in contact. The time intervals, however, are proportionately the same as recorded for the process in agar medium, and the speed of the reaction can be attributed to the relatively greater ease of movement of substances in water by currents than in agar through diffusion alone.

It is also significant that under both cultural conditions no change in the sequence of events has ever been observed.

Incompatibility in interspecific matings of a. ambisexualis and a. bisexualis.—Incompatibility with failure of gametic union in the reciprocal matings between A. bisexualis and A. ambisexualis furnishes additional evidence for the hormonal mechanism. The sexual reaction never proceeds to completion in either of the two matings; the points at which the reaction stops in the two instances indicate definite specificity of two of the hormones involved.

In matings of A. ambisexualis  $\mathcal{P} \times A$ . bisexualis ô incompatibility appears to be due to a failure of the agent which brings about the delimitation of the antheridium in a normal mating. It has been repeatedly seen that when this mating is made in agar or in water the first three stages of the sexual reaction occurs as in matings of perfectly compatible strains. The production of antheridial hyphae on the ô plant, the subsequent formation of oogonial initials on the 9, and the attraction of the antheridial hyphae to the oogonial initials are all normal. At this point, however, the coordinating mechanism breaks down and no antheridia are delimited, although the 3 initials are wrapped around the 9 organs (fig. 23). This suggests specificity of some substance produced by the oogonial initial which is necessary for the delimitation of the antheridium. It will be shown in the second paper of this series that this is the case, and this reaction to a specific substance is coupled with a thigmotropic response of the antheridial hyphal tip to solid substances.

This reaction further supports the concept that a substance, produced by the antheridium, is effective in the differentiation of the oogonium. For here, although the antheridial branches are applied to the wall of the oogonial initial as in normal reproduction, subsequent development of the  $\mathfrak{P}$  sexual organs

fails to occur. If the delimitation of the oogonium were simply a thigmotropic response to the presence of antheridial hyphae applied to its wall, this differentiation would take place. Apparently a substance produced by the antheridium in contact with the oogonial initial is necessary for the delimitation of the latter.

Incompatibility is again seen in the reciprocal mating, A. ambisexualis  $\delta \times A$ . bisexualis  $\circ$ . This reaction, however, differs markedly from that observed in the preceding mating. Antheridial branches are produced in profusion on the & hyphae, but there is no reaction in the  $\mathcal{P}$  in response to the extensive production of these organs and, presumably, the secretion of a large amount of the substance which normally would bring about the formation of oogonial initials. In water matings of these two plants antheridial hyphae are attracted to the vegetative hyphae of the 2 and become intricately entwined about these filaments (fig. 24). There is no reaction in the female except for the production of structures which at first appear to be oogonial initials but which later become differentiated not as oogonia, but as "resistant spores." These resistant spores were first described by Weston (1917) and have since been shown by the writer (1936) to be resistant vegetative structures characteristic of the  $\circ$  strains of A. bisexualis. The number of these spherical bodies is increased by the presence of the partially compatible 3 plant, and in such a mating they are occasionally produced on lateral branches, while in single culture they are borne only in a terminal position on the vegetative hyphae.

When this interspecific mating is made on agar, the reaction is the same as in water except for one significant difference. The directional growth of antheridial branches toward the vegetative hyphae of the  $\mathfrak P$  and the characteristic twining around the  $\mathfrak P$  hyphae occur only near the surface of the agar, presumably where a supply of oxygen is available. The formation of antheridial branches, however, is not limited to this region, approximately the same quantity being produced at all levels in the substrate.

The production of antheridial branches by the  $\delta$  indicates that the primary specific substance of A. bisexualis  $\mathfrak P$  is capable of bringing about the same specific reaction caused by the primary substance of A. ambisexualis  $\mathfrak P$ . The attraction of the antheridial branches to the vegetative hyphae of the  $\mathfrak P$  indicates further that this substance is also effective in causing directional growth of the antheridial hyphae, which is normally (i.e., in compatible matings) controlled by a substance diffusing from the oogonial initial. The antheridial branches do not respond further by the delimitation of antheridia as in the presence of oogonial initials of a  $\mathfrak P$  plant of the same species.

Analysis of the incomplete sexual reactions between A. ambisexualis and A. bisexualis indicates that high specificity of certain of the sexual hormones is responsible for the failure of the process. The logical conclusion to draw from these observations is that complete incompatibility would result when the  $\delta$  is incapable of responding to the primary substance secreted by the vegetative mycelium of the  $\mathfrak{P}$ .

Effects of variations in the composition of the medium.—In an attempt to determine the range of substances necessary for the accomplishment of the sexual process, matings of  $\delta$  and P plants have

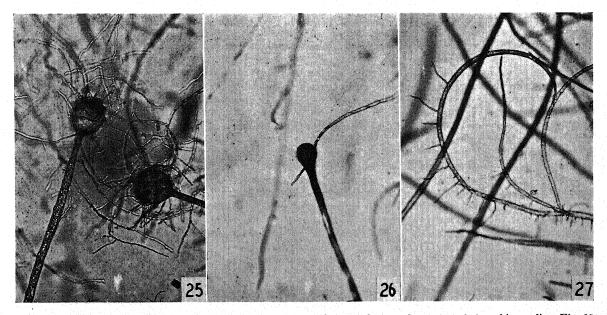


Fig. 25-27. Effects of variations in the composition of the medium on the sexual reaction of A. ambisexualis.—Fig. 25. Insufficient quantity of Fe+++ results in failure of oogonial delimitation.  $\times 80$ .—Fig. 26. PO<sub>4</sub>--- deficiency results in failure of attraction of antheridial branches to oogonial initials.  $\times 40$ .—Fig. 27. Certain nutritional conditions cause failure of sexual reaction at completion of first stage.  $\times 40$ .

been carried out on a large number of agar media. While these experiments have not given the results hoped for, certain facts have been observed which support the concept of coordination by hormones in the sexual process. By manipulating the composition of the semi-solid substrate on which the plants were mated, it has been found that until the time of oogonial delimitation the sexual reaction can be stopped at any desired point.

When the amount of iron in the medium is decreased, a significant effect on the reproductive process is brought about. The reaction is at first normal, antheridial hyphae being produced in large numbers. The second stage of the reaction, the formation of oogonial initials, follows as in an ordinary mating. The third and fourth stages, attraction of antheridial hyphae to the oogonial initials and subsequent delimitation of antheridia, occur in the typical order and manner. At this point the reaction stops, the oogonial initial failing to form a basal wall to delimit the  $\mathcal{P}$  gametangium (fig. 25).

To explain this phenomenon two possibilities are suggested: (1) that a certain amount of iron, which is here present in insufficient concentration, is needed by the antheridium for the elaboration of the oogonial delimiting agent, (2) that a greater concentration of iron than is present is required by the oogonial initial before a basal wall can be formed.

A second similar effect is seen when the amount of phosphate in the medium is decreased. Ordinarily KH<sub>2</sub>PO<sub>4</sub>, in concentration of M/30,000, is added to the medium, but here this salt is omitted, potassium being furnished in equal amount as KCl. Of course phosphate is present in the starch which is used as a carbohydrate source, but the omission of the amount which is normally added causes interesting and significant changes in the sexual reaction. The initial reaction is normal with the production of large numbers of antheridial hyphae by the 3 mycelium. The next stage is also apparently normal, for oogonial initials are formed in considerable quantity. At this point, however, the coordination of the sexual processes is interrupted, for the antheridial branches are not attracted to the oogonial initials. Figure 26 presents this phenomenon well, for it shows an antheridial branch which touches an oogonial initial, avoids it, and continues growing, an event which has never been seen in a normal mating. This suggests that phosphate in greater concentration is necessary for the normal reaction brought about by a substance secreted by the oogonial initial. Again, as in the preceding case, no evidence is present to indicate whether this effect depends on the production or the utilization of the substance. The experiment, however, gives supporting evidence for the attraction of the antheridial branches to the oogonial initial by a substance secreted by the ? initial itself rather than a second response to a substance produced at all times by the 2 mycelium.

The disruption of the sexual process at the end of the first & reaction has also been observed. Of a large number of media tried three were found which would support more or less vigorous growth but on which the only reaction between the two mycelia was the production of antheridial branches. No further development was seen in either 3 or 2. These media, prepared with Pyrex distilled water containing low concentrations of various salts (KH<sub>2</sub>PO<sub>4</sub>, M/3×10<sup>4</sup>; MgSO<sub>4</sub>, M/8×10<sup>4</sup>; CaCl<sub>2</sub>, M/10<sup>5</sup>; FeCl<sub>3</sub>, M/10<sup>6</sup>; and ZnSO<sub>4</sub>, M/10<sup>7</sup>), are as follows:

- 1. Cysteine-HCl
   .0.05%
   2. Starch
   .0.3%

   Sodium Butyrate
   0.05%
   i-Inositol
   .0.1%

   i-Inositol
   .0.05%
   Glycine
   .0.05%

   Agar
   .2.0%
   K<sub>2</sub>S
   Trace

   Agar
   .2.0%

   Water
   Water

In media 2 and 3,  $K_2S$  and cysteine-HCl were added to provide utilizable sulfur, these plants as shown by Volkonsky (1933) being incapable of reducing the sulphate radical.

Å few media were tested which were apparently unable to support the sexual reaction in any of its stages. On a medium composed of 0.3 per cent starch, 0.1 per cent i-inositol, 0.1 per cent cysteine-HCl, 2.0 per cent agar, and the salts listed above, the reaction is normal, a large number of mature sexual organs and fertilized eggs being formed. In media having cystine or methionine substituted for the cysteine-HCl in the above formula, vigorous and healthy growth of the mycelia occurs, but there is no reaction between the ♂ and ♀ plants.

Since compatible mycelia have always been observed to give a strong normal sexual reaction in lentil-extract agar, this medium has been used as a control in all these experiments. Lentils are known to contain two substances often shown to be nutritional requirements for a number of plants: vitamin  $B_1$  and inositol. Of these, vitamin  $B_1$ , when added to various media, causes no appreciable change in the vigor of either growth or sexual reactions of the plants. This differs from the condition found in Phycomyces Blakesleeanus by Schopfer (1931), who showed that the addition of this vitamin increased the intensity of the sexual reaction between (-) and (+) strains of that species. The possibility of the reaction being influenced by the other growth component, inositol, has likewise been investigated, but the results are not unquestionably definite.

Hydrogen-ion concentration was early suspected of being an important factor in the reproductive process. To test this possibility  $\delta$  and  $\mathfrak P$  plants were mated on a series of starch-peptone-inositol agars adjusted to pH values of 3.7, 4.5, 5.0, 5.5, 6.0, 7.0, and 7.5 by means of potassium phosphate buffers (M/150, total phosphates). Growth was equally vigorous in all cases; similarly, formation and matura-

tion of sexual organs did not vary appreciably at the different levels of hydrogen-ion concentration.

THE HORMONAL MECHANISM.—From the different lines of evidence presented above it is obvious that a well-developed mechanism is operative in initiating and coordinating the different stages of the sexual process in matings of ô and ♀ plants of Achlya ambisexualis and A. bisexualis. The sequence of the events in the process and the time intervals at which they take place indicate that this coordinating mechanism involves diffusible substances, and the pattern of incompatibility in interspecific matings between these two species as well as the manner of disruption of the process by changes in the composition of the medium indicates that these substances are four in number and that they are characterized by a certain degree of specificity. Furthermore, this body of evidence points to the probability that the sexual reaction in both these species is under hormonal control, at least until the time of the delimitation of the oogo-

During the time which elapses between the approximation of the  $\delta$  and  $\mathfrak P$  mycelia and the formation of the basal wall of the oogonium, two substances are produced by the  $\mathfrak P$  which bring about specific responses in the  $\delta$ , and two are produced by the  $\delta$  which induce specific reactions in the  $\mathfrak P$ . These hormones, the points of their origins, and their specific activities have been reported in a preliminary note (Raper, 1939) from which table 1 is taken.

The reactions brought about by these hormones constitute a chained reaction with each of the different stages in the reproductive process being dependent on the preceding ones.

The rôle of these hormones as coordinators in this process can easily be seen. Hormone A is the only one of these substances which is not produced by specialized and localized organs. It is apparently secreted at all times by the vegetative mycelium of the female. Antheridial branches are produced only in response to the presence of hormone A. The ô initials thus formed, in turn, produce a second specific substance, hormone B, which is required for the next stage of the sexual reaction—namely, the formation of oogonial initials. The oogonial initials, once they reach maturity, produce a third substance, hormone

C, which has two functions: (1) causing directional growth of the antheridial branches toward the point of its production, the  $\,^{\circ}$  initials, and (2) bringing about the delimitation of antheridia at the tips of the antheridial branches once they are applied to the walls of the oogonial initials. Finally, a fourth substance, hormone D, is secreted only by the delimited antheridium, and it brings about the formation of a basal wall which separates the oogonium from the parent  $\,^{\circ}$  hypha.

In the normal course of events in matings, either in water or in a semi-solid substrate, this mechanism of hormonal coordination induces the formation of the various sexual organs only when they may be most effective. In certain cases, however, as shown in the inter-specific matings and the nutritional experiments, it is possible to bring about the breakdown of the system with consequent decrease, or complete failure, in gametic union and the production of fertilized eggs.

The term "hormones" is here applied to the specific sexual substances of *Achlya* in accord with Huxley's (1935) classification of hormones.

Hormones were first defined by Bayliss and Starling (1904) as substances which, being produced in one part of the animal body, are carried by the blood stream to another part where they bring about a specific physiological reaction. In their monograph, Phytohormones, Went and Thimann (1937) revised this definition to include the auxins, defining a hormone as a chemical substance which, being produced in one part of an organism, is transported to another where its activity is manifested. It was pointed out logically enough by Huxley that whether or not a chemical substance is a hormone should depend on its activity rather than the mechanical mode of its transportation. His classification separates hormones into two groups: A, "regional activators" and B, "distance activators." In the second group he places those substances, which, transported by diffusion within the body of a single organism or between different individuals, bring about specific responses. In this category would belong the first three substances defined above, while the fourth, hormone D, which is not diffusible through long distances,

TABLE 1.

Hormone	Produced by	Affecting	Specific action(s)
A	Q-Vegetative hyphae	∂-Vegetative hyphae	Induces formation of antheridial branches
В	&-Antheridial branches	Q -Vegetative hyphae	Initiates the formation of oogonial initials
c	Q-Oogonial initials	3-Antheridial branches	<ol> <li>Attracts antheridial branches</li> <li>Induces, in connection with a thigmotropic response, the delimitation of antheridia</li> </ol>
D	∂-Antheridia	♀-Oogonial initials	Brings about the delimitation of the oogonium by the formation of a basal wall

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could perhaps best be included in the group, "chemical differentiators" of the "regional activators."

### SUMMARY

During the course of an investigation of the sexuality in Achlya, a new species was found and is here described as A. ambisexualis. Ten isolates of this plant, collected by the writer, are separated on the basis of morphology of vegetative mycelium and sexual organs into three varieties: type, var. abjointa, and var. gracilis. The sexuality of the plant agrees with that of other partially separate-sexed species of the watermolds which have been described as heterothallic.

The successive stages in the sexual reaction of A. ambisexualis and A. bisexualis are described in detail. Until the time of P gametangial differentiation, five stages are recognized: (1) production of antheridial hyphae on P mycelium, (2) formation of oogonial initials on P, (3) directional growth of antheridial branches to P initials and the application of the tips of the P initials to the wall of the P organ, (4) the delimitation of antheridia at the tips of the

antheridial branches in contact with the  $\varphi$  initial wall, and (5) the formation of a basal wall delimiting the oogonium from the parent  $\varphi$  hypha.

The rigid sequence of events and the constantly proportionate time intervals between these stages indicate a well developed coordinating mechanism.

Incompatibility in inter-specific matings between A. ambisexualis and A. bisexualis is apparently the result of interruptions in the coordinating mechanism. The reaction differs in the two reciprocal matings.

By varying the chemical composition of the medium, disruption of the sexual process at times coinciding with the initiation of the several stages indicates specific substances as the coordinating agents.

On the basis of these facts, a hormonal system is outlined. Four specific substances are involved: two from the  $\delta$  and two from the  $\varphi$ , each bringing about specific responses in the activity of the opposed mycelium.

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# THE SIGNIFICANCE OF WOOD ANATOMY IN THE TAXONOMY OF THE JUGLANDACEAE <sup>1</sup>

Charles Heimsch, Jr., and Ralph H. Wetmore

Manning (1938a) has recently made a comparative morphological investigation of inflorescences of the various members of the Juglandaceae. He finds that the inflorescences of the family are based on a fundamental pattern though showing varying degrees of reduction along certain definite lines from a primitive type. This primitive type is a terminal androgynous panicle consisting of a central pistillate catkin surrounded by several staminate branches or catkins. In Manning's opinion, the genera Alfaroa, Engelhardtia, and Platycarya exhibit this primitive type, whereas the genera Juglans, Carya, and Pterocarya possess specialized or derived types of inflorescences. His conclusions were founded on morphological rather than anatomical considerations.

That studies of the secondary xylem in the dicotyledons may aid the phylogenist in his attempts to establish natural relationships among certain families of Angiosperms has been shown by Vestal (1937), Tippo (1938), and Taylor (1938), students from these laboratories. In these investigations involving taxonomic groupings of families attempts were made to correlate xylem structure with existing systems of classification, and their findings indicate an interestingly high degree of correlation between the two. So much is this the case that groups which fail to show this correlation are suspected of being erroneously placed in the system being examined.

Bailey and his students have established certain lines of structural specialization in dicotyledonous xylem. Bailey and Tupper (1918) obtained significant data regarding the classification and length of vascular elements. Frost (1930a, 1930b, 1931), by statistical methods, after a survey involving the majority of the dicotyledonous families, revealed the trends of specialization of the vessel segment. Using Frost's results as a basis, Kribs (1935) found a high degree of statistical correlation between the type of wood rays and the degree of specialization in the vessel segment. By use of similar methods, Kribs (1937) correlated vertical parenchyma patterns with primitive and advanced types of vessel members.

Although the previous attempts to use these anatomical tools have had as their central theme the determination of relative positions of families in natural taxonomic groupings, evidence was also obtained that these anatomical principles would apply equally well to considerations within the specific family, providing a significant range of structural variability exists in the family.

A detailed description of the anatomy of the secondary xylem of the Juglandaceae was published by Kribs in 1927, based on the limited material available to him. With these lines of specialization in mind, and a fairly complete representation of the genera with which to work, the present investigation was undertaken in order to determine to what extent the findings based on xylem anatomy of the Juglandaceae would accord with those obtained from a study of the organization of the inflorescences.

MATERIALS AND METHODS.—For this investigation the microscopic slides, the dried, and the preserved wood in the Harvard Collection were at the writers' disposal. Through the courtesy of Professor Samuel J. Record of the Yale School of Forestry, additional specimens were obtained. Material was also collected from members of the family growing in the Arnold Arboretum.

Before additional slides were made, it was found necessary to soften the material in hydrofluoric acid. When embedding was necessary, celloidin was used, following the standard technique of Wetmore (1932). The living material was fixed in formacetic-alcohol, washed in tap water, softened and embedded. Sections 10 micra thick were made, stained with safranin and Heidenhain's iron-alumhaematoxylin, and mounted permanently in Canada balsam or diaphane.

Macerations were made of representative material of all the genera, using equal amounts of 10 per cent chromic and 10 per cent nitric acids at a temperature of 60°C. for 48 hours. Following thorough washing, the separated elements were stained with water-soluble nigrosine and dehydrated in rapidly increasing percentages of ethyl alcohol. Permanent mounts were made using diaphane as a mounting medium.

Preliminary examinations showed that when 50 measurements of the vessel member lengths and of fiber-tracheid lengths were used, these followed the normal curve of distribution; thus, this number of length measurements was averaged for the respective elements for each species. From these the averages for the genera were derived. For vessel diameters 25 random measurements were averaged for each species represented, except for those species which were ring-porous or tended toward ring-porosity, then only the larger pores were measured.

The values for the angle of the end wall were obtained by taking 10 random measurements of each species with a camera-lucida. These were averaged, and from these the averages for the genera were computed. Preliminary figures concerning ray height were not significant, and this can be attributed to ontogenetic changes in the form and character of the wood rays. With increased knowledge concerning the ontogeny of the various types of wood rays, statistical treatment of the characters may prove to be significant.

All photographs were taken on DC Ortho plates with Zeiss lenses and Zeiss photomicrographic equipment.

<sup>&</sup>lt;sup>1</sup> Received for publication June 19, 1939.

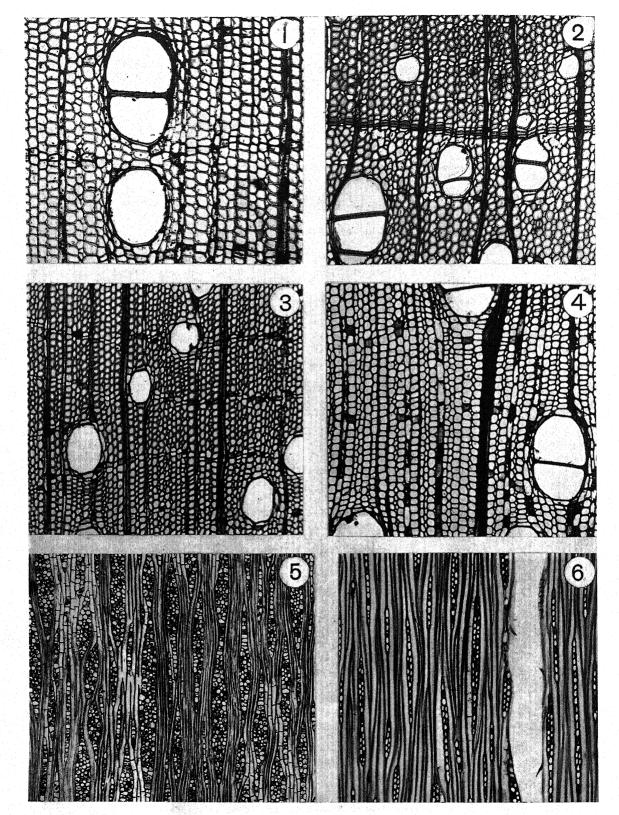


Fig. 1-6.—Fig. 1. Alfaroa costaricensis Standl. Transverse section showing thin-walled fiber-tracheids, thin-walled angular to round pores, and banded apo-paratracheal parenchyma. ×155.—Fig. 2. Pterocarya Paliurus Bat. Trans-

Anatomical description of the genera.—Alfaroa (fig. 1, 8, 11, 13).—The one species, A. costaricensis Standl., is native to Central American highlands. Growth rings are present. The main ground mass of imperforate cells consists of thin-walled fiber-tracheids; as might be expected, these fibertracheids of the late wood become progressively thicker walled. Arcs of gelatinous fibers are present in the twigs. Vessel pores are chiefly solitary or in radial multiples of 2-6 with a few clusters and short chains (fig. 1). The wood is definitely diffuse-porous. The vessels are thin-walled with angular to rounded outlines and possess a few thin-walled tyloses. Vessel perforations may be either scalariform or porous with a higher percentage of the scalariform type than elsewhere in the family (fig. 8). The bars range from one to twelve or more in number, and the apertures are completely bordered.

Intervascular pitting is circular-bordered and alternate (fig. 8), a feature in distinct contrast to the predominate gash-like vessel-ray (fig. 11) and vessel-parenchyma pitting. The rays are heterogeneous I (Kribs, 1935), and the multiseriate portions of the multiseriate rays are from 2-3 cells wide (fig. 13). Parenchyma occurs in fine concentric bands, one to two cells in width. There is also scanty vasicentric and terminal parenchyma. Although the parenchyma is not concentrated about the vessels, one or more vessels are infrequently contacted by the bands in their tangential extension. This places the parenchyma pattern intermediate between distinct apotracheal and distinct paratracheal types (Chalk, 1937), which fact seems to justify the use of the term apoparatracheal in describing the parenchyma pattern occurring here. Rays frequently contain small crys-

Engelhardtia (fig. 3, 4, 9, 10, 12).—The genus consists of 11 species from southeastern Asia, Mexico, and Central America. Of the 8 species investigated, only two, E. Fenzellii Merrill² and E. pterocarpa Standl.,³ show definite growth rings. However, the other specimens give evidence of a periodic slowing down in growth processes in the form of thickened fibers, but no definite rings are present. The imperforate tracheary elements are of the fiber-tracheid category with slightly thicker walls than those of Alfaroa. Gelatinous fibers occur in E. pterocarpa. Though vessels are predominately either solitary or in radial multiples, the relative percent-

<sup>2</sup> Manning (1938a) indicates that *E. chrysolepis* Hance, *E. Fenzellii* Merrill, and *E. formosana* Hayata are synonyms.

<sup>3</sup> Standley (1927) suggests that *E. pterocarpa* Standley and *E. Oreomunnea* C. DC. are synonyms.

age of each may vary considerably. Invariably a few pore clusters are present. All species are diffuse-porous. Vessels are angular to round in outline, with the tendency toward roundness more pronounced in some species (fig. 3, 4). Vessel walls are thin, and thin-walled tyloses were present in about half of the species.

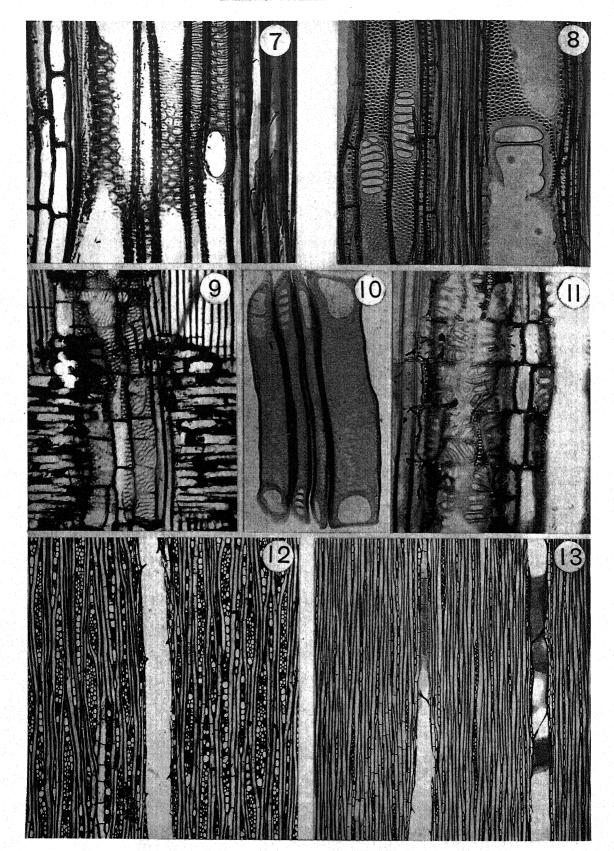
Vessel perforations are either scalariform or porous (fig. 10). The scalariform perforations occur chiefly in the smaller segments and are found in all species. The bars range from one to nine or more in number, and, contrary to Kribs' statement (1927), there is no distinction between the American and the Asiatic species in this regard.

Intervascular pitting is circular-bordered and alternate. Vessel-ray and vessel-parenchyma pitting varies from small half-bordered pit-pairs in certain species to small half-bordered and gash-like pit-pairs in other species (fig. 9). Similarly the rays vary from heterogeneous IIA in some species to heterogeneous IIB in others, with some species transitional between the two ray types (fig. 12). Multiseriate rays are from 2-4 cells wide.

E. spicata Blume has diffuse, diffuse-in-aggregates, banded apotracheal and scanty vasicentric wood parenchyma (fig. 4). The pattern is irregular with the emphasis on diffuse-in-aggregates and scanty vasicentric. E. spicata Blume, var. aceriflora Koord. and Valet. has a more regular parenchyma pattern with the narrow apotracheal banded condition more pronounced; however, the diffuse-inaggregate condition is present. These descriptions are in general agreement with those of Janssonius (1936) for the above species. In the remaining species, the apo-paratracheal banded condition predominates (fig. 3). There is, in addition, a tendency for the parenchyma to be irregularly clustered about the vessels, forming caps, with the narrow bands extending laterally from these. Diagonal connections between some bands occur. In all species except E. chrysolepis and E. Fenzellii some parenchyma exists in diffuse aggregates. Those species with growth rings possess terminal parenchyma. Crystals were found in the vertical parenchyma of E. formosana, E. pterocarpa, and E. Oreomunnea.

Pterocarya (fig. 2, 6).—The genus is comprised of 8-9 species; these are found in Trans-Caucasia, China, and Japan. Of the 5 species investigated, all showed growth rings. Imperforate tracheary elements are thin-walled fiber-tracheids except at the terminus of each growth ring where a narrow band of thick-walled elements occurs (fig. 2). Twigs of P. stenoptera C. DC. and P. hupehensis Skan. show gelatinous fibers. Vessel pores are chiefly solitary or

verse section showing growth ring; fiber-tracheids with slightly thickened walls (thick walled in late wood); thin-walled, angular to round pores; banded apo-paratracheal parenchyma, and terminal parenchyma. ×92.—Fig. 3. Engelhardtia Fenzellii Merr. Transverse section showing fiber-tracheids with slightly thickened walls; thin-walled, angular to round pores; and banded apo-paratracheal parenchyma. ×92.—Fig. 4. Engelhardtia spicata Bl. Transverse section showing distribution of elements, particularly diffuse, diffuse-in-aggregate, and scanty vasicentric vertical wood parenchyma. ×92.—Fig. 5. Platycarya strobilacea Sieb. and Zucc. Tangential section showing wood rays, vertical parenchyma, and small vessels associated with vasicentric tracheids. ×48.—Fig. 6. Pterocarya stenoptera C. DC. Tangential section showing nearly homogeneous rays. ×92.



in short radial multiples with a few clusters. All species are distinctly diffuse-porous except P. Paliurus Bat. which tends to be ring-porous. Vessels of P. stenoptera C. DC. are angular in outline, all others angular to round. All vessels are thin-walled and a few thin-walled tyloses are present.

Vessel perforations are all porous, and intervascular pitting is alternate. Vessel-ray and vessel-parenchyma pitting consists of small half-bordered pitpairs. Rays are heterogeneous IIB, and the multiseriate rays are from 2-4 cells wide (fig. 6). Parenchyma is of apo-paratracheal bands, mostly onecell wide, these bands being quite regular (fig. 2). Accompanying this pattern is scanty vasicentric pa-

renchyma in all species.

Juglans (fig. 14, 17, 19).—The existing species of Juglans are found in China, Japan, India, Persia, United States, Mexico, Central America, West Indies, and the Andean region of South America from Colombia and Venezuela to Argentina. Of the 14 species investigated, J. pyriformis Liebm. from Salvador showed no growth rings, and J. insularis Griseb. from Cuba showed them only faintly. All other specimens possess distinct growth rings. The imperforate tracheary cells are all fiber-tracheids. As Kribs indicates (1927), J. cinerea L. (fig. 19), J. mandschurica Maxim., and J. Sieboldiana Maxim. tend to have vascular elements with thinner walls than those of the remaining species (fig. 14, 17). As in Pterocarya, the growth rings are terminated by narrow bands of elements with thickened walls (fig. 19). A few species show gelatinous fibers.

Vessel distribution follows that of the previously described genera with emphasis on the solitary and multiple arrangements with occasional clusters. In general the genus is diffuse-porous, but its members exhibit a distinct tendency toward ring-porosity. In most cases there is a gradual diminution of vessel diameter from the early to the late wood of a season's growth. The vessels possess angular to rounded outlines with a greater tendency toward roundness than was present in the above genera. Vessel walls are thin; those forms which have thinner-walled fibertracheids likewise have vessels with thinner walls. Tyloses are generally present, varying from few to many; in all cases they are thin-walled.

Vessel perforations are strictly porous, and intervascular pitting is uniformly circular bordered and alternate. Vessel-ray and vessel-parenchyma pitpairs are small and half-bordered. Rays are generally heterogeneous IIB, but in J. nigra L. and J. Sieboldiana they closely approximate homogeneity. Multiseriate rays range from 2-5 cells in width.

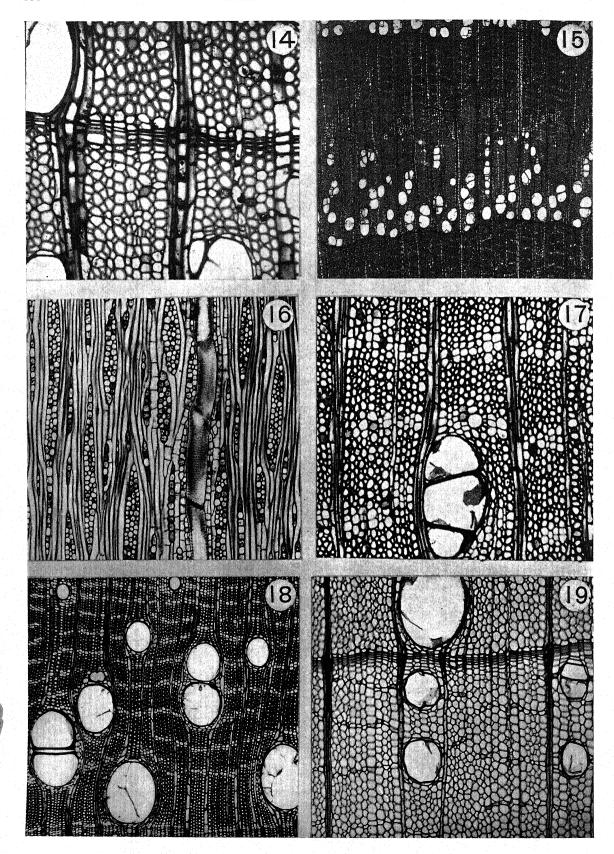
In all but three species there is some parenchyma that is diffuse or diffuse-in-aggregates. In J. californica S. Wats., J. mandschurica, J. nigra, and J. rupestris Engelm. the above parenchyma patterns predominate (fig. 14). In these cases there is always a variable amount of vasicentric parenchyma present. In most of the species there is a definite banding of the parenchyma; in J. australis Griseb., J. boliviana Dode, and J. Sieboldiana, the banding is more independent of the vessels than in J. cinerea, J. colombianensis Dode, J. insularis, J. neotropica Diels, and J. pyriformis Liebm. (fig. 17). Because of the association with the vessels in these latter species, it seems that the bands can most properly be called apo-paratracheal. Terminal parenchyma is present in all species with growth rings (fig. 19). Crystalliferous parenchyma strands are present in most of the species.

Carya (fig. 16, 18, 20, 21).—The 18 or 19 species of this genus are found from Canada to Mexico in the eastern half of the United States, with one species in China, and one species in Indo-China. All the species investigated show growth rings. Imperforate tracheary elements are fiber-tracheids, but in some cases the pit borders are exceedingly minute. Gelatinous fibers are present in a few species. Pores are chiefly solitary and in radial multiples of 2-4 with occasional short chains and clusters. All species are distinctly ring-porous (fig. 18), although C. tonkinensis Lecomte, an Asiatic species, does not show the extreme size difference between the vessels of the early and late wood that are found in the American species (fig. 21). Vessel outlines are exclusively round, and the walls are thickened over the vessel walls of the previously described genera (fig. 18). Especially prominent in this regard are the extremely thickened walls of the small late-formed vessels in the seasonal growths. Numerous thinwalled tyloses are characteristic of the genus, and they occur almost entirely in the large vessels.

Vessel perforations are exclusively porous, and intervascular pitting is crowded and alternate. Vessel-ray and vessel-parenchyma pitting consists of small half-bordered pit-pairs. Rays are heterogeneous IIB (fig. 16). As Kribs states (1928), those of C. tonkinensis are more heterogeneous than those of the American species (fig. 20). The multiseriate rays range from 2-5 cells in width.

Generally, in Carya, the parenchyma is distinctly banded, the bands being apo-paratracheal and associated with vasicentric parenchyma in varying amounts (fig. 18, 21). However, most species show some diffuse and diffuse-in-aggregate parenchyma

Fig. 7-13.—Fig. 7. Platycarya strobilacea Sieb. and Zucc. Radial section showing porous perforation plate and vessels and vasicentric tracheids with spiral thickenings. ×285.—Fig. 8. Alfaroa costaricensis Standl. Radial section showing types of scalariform perforation plates, and alternate, circular bordered, intervascular pitting. ×148.—Fig. 9. Engelhardtia serrata Bl. Radial section showing variation in vessel-parenchyma pitting. ×121.—Fig. 10. Engelhardtia Fenzellii Merr. Macerated vessel elements resulting from same cambial initial showing variations in perforation plates. ×145.—Fig. 11. Alfaroa costaricensis Standl. Radial section showing gash-like vessel-ray pitting. ×225.—Fig. 12. Engelhardtia spicata Bl. Tangential section showing heterogeneous rays and oblique end walls of vessels. ×92.—Fig. 13. Alfaroa costaricensis Standl. Tangential section showing decidedly heterogeneous rays, intervascular pitting, and oblique end walls of vessels. ×48.



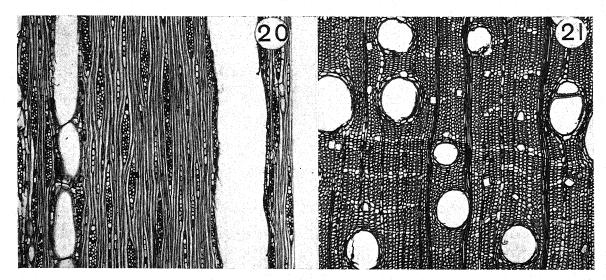


Fig. 20-21.—Fig. 20. Carya tonkinensis Lecomte. Tangential section showing heterogeneous wood rays, slightly oblique end walls of vessel elements, and crystals in wood parenchyma. ×48.—Fig. 21. Same, transverse section showing growth ring, pores with thickened walls, and banded apo-paratracheal with terminal parenchyma, both types with crystals. ×48.

in the spring wood. Crystals occur in swollen parenchyma cells, being most abundant in *C. tonkinensis* (fig. 20, 21).

Platycarya (fig. 5, 7, 15).—P. strobilacea Sieb. and Zucc., the one species, is confined to China and Japan. The wood shows well-defined growth rings (fig. 15). Fiber-tracheids compose the greater part of the wood, and those of the late wood have thickened walls over those of the early wood. Gelatinous fibers are also present. The wood is ring-porous. Large vessels are solitary, in short radial multiples, chains or clusters, whereas the smaller vessels and the vascular tracheids with which they are associated are in a flamelike arrangement. These small vessels and the vascular tracheids have spiral thickenings in the secondary walls (fig. 7). Large vessels have moderately thickened walls and tend to be round in outline. Small vessels are thinner-walled and angular. A few thin-walled tyloses are present in the large vessels.

Vessel perforations are exclusively porous. Intervascular pitting is alternate, and vessel-ray and vessel-parenchyma pitting consists of small half-bordered pit-pairs. Rays are heterogeneous IIB, and the multiseriate rays are often very high (fig. 5). Likewise relatively long uniseriate tips of more or less upright cells are common to the multiseriate rays. The multiseriate rays range from 2-8 cells in

width, thus they are the widest in the family. Sections of twigs near the pith indicate that these widths are the result of ontogenetic increase in cell numbers. Uniseriate rays are few in number and are from low to very high.

In the early wood, parenchyma is diffuse, diffuse-in-aggregates and scanty vasicentric (fig. 15). This gives way, in the late wood, to a banded apotracheal condition. It could not be properly determined whether or not terminal parenchyma was present, but from all indications it is. Rhomboid crystals occur in ray cells.

General descriptions it is evident that certain anatomical descriptions it is evident that certain anatomical characters are more or less constant throughout the family. Among these are the characteristic fibertracheids of the family. However, the thickness of the walls of these elements varies. Gelatinous fibers occur in every genus. Solitary pores with varying percentages of short radial multiples are the rule. Intervascular pitting is always alternate, and thinwalled tyloses are universally present.

In addition, the pattern of the wood parenchyma varies but little from genus to genus. Hyde (1925) and others have referred to tangential banding of parenchyma as being like that of the Juglandaceae. In each genus the banded nature of the parenchyma predominates, although variations from this pattern

Fig. 14-19.—Fig. 14. Juglans rupestris Engelm. Transverse section showing growth ring, fiber-tracheids with moderately thickened walls (thick walled in late wood), angular to round pores with slightly thickened walls, and diffuse-in-aggregates, scanty vasicentric and terminal wood parenchyma. ×157.—Fig. 15. Platycarya strobilacea Sieb. and Zucc. Transverse section showing growth rings, small vessels with vasicentric tracheids in flames, and lack of banded parenchyma in early wood with distinct banding in late wood. ×15.—Fig. 16. Carya pecan C. K. Schneider. Tangential section showing slightly heterogeneous rays and small vessels. ×92.—Fig. 17. Juglans pyriformis Liebm. Transverse section showing wide banded apo-paratracheal parenchyma. ×92.—Fig. 18. Carya alba Nutt. Transverse section showing large and small pores, and banded apo-paratracheal parenchyma. ×48.—Fig. 19. Juglans cinerea L. Transverse section showing growth ring, thin-walled fiber-tracheids (thick walled in late wood) thin-walled, angular to round pores, terminal and banded apo-paratracheal parenchyma.

do occur. A few species only show a preponderance of diffuse or diffuse-in-aggregate parenchyma. In practically every slide which shows growth rings terminal parenchyma<sup>4</sup> is a counterpart.

It is obvious that the several characters with limited variation contribute no evidence with reference to the anatomical specialization of the individual genera. Also it is noteworthy that the variation in certain characters is dependent upon the environment—i.e., diffuse-versus ring-porosity; and proper allowances for this fact need to be made in dealing with relative specializations. However, it will be seen that in the Juglandaceae these instances which merit special considerations do not detract from the general scheme of xylem organization and thus do not impose limitations upon ideas concerning the anatomical specialization of the genera.

It will be recalled that Manning ascribes a primitive type of inflorescence to the genera Alfaroa, Engelhardtia, and Platycarya. The thin-walled fibertracheids of Alfaroa are more primitive than those with thicker walls in the other genera. Diffuse-porosity is less advanced than is ring-porosity (Frost, 1930a). It is commonly accepted that the scalariform vessel perforation is more primitive than the porous perforation; thus, in this regard, Alfaroa is the most primitive genus of the family. In their thin walls and angular outlines, the vessels also reveal primitive characteristics. Likewise, the heterogeneous I type of ray present in the genus is the most primitive type of the family. Thus it is quite evident that the secondary xylem of Alfaroa is rather primitive. This is in direct agreement with the results of the inflorescence studies. Additional evidence for the primitive status of Alfaroa is brought forth by Manning (1938b) in that he relegates the stigmas to a low position when compared with those in the other genera of the family.

<sup>4</sup> Terminal parenchyma is defined in the Glossary of the Committee on Nomenclature of the International Association of Wood Anatomists (1933) as being "aggregated wood parenchyma forming a more or less continuous layer of variable width at the close of a season's growth." It appeared, however, in the majority of cases to be a part of the spring growth but on the face of the old growth ring. Chowdhury (1936) has suggested that such parenchyma be termed "initial parenchyma" rather than terminal.

Since these first formed elements in the spring growth are parenchymatous, it was thought that perhaps the septations in the fusiform daughter cells might occur in the fall with further differentiation delayed until spring. If this proved to be the case, the use of the term "terminal" would then be justified. Material of a few members of the family was collected in late November with considerable snow on the ground, and from all indications growth had ceased. Upon examination after sectioning, contradictory evidence was obtained. In Pterocarya Rehderiana C. K. Schneider, the majority of the cambial daughter cells, as well as cells which were wholly or partially differentiated, showed no cross walls. In Juglans cinerea, however, the majority of these late-formed elements showed septations. This only serves to indicate that specific differences as well as specific environmental responses may be involved. More material, collected at the proper time, must be investigated to determine which, if either, of these cases would hold true for the remainder of the family.

Engelhardtia as a genus agrees markedly with Alfaroa in its diffuse-porosity. Although the vessels tend to lose their angularity, they remain thinwalled. The fiber-tracheid walls are also relatively thin. In addition, scalariform vessel perforations are present, although they are less abundant than in Alfaroa. Manning (1938a) states that some species of Engelhardtia show considerable advance in inflorescence type and that (1938b) both primitive and advanced types of stigmas are present in the genus. This more or less harmonizes with the variation found in vessel perforation types. In this connection, a figure from E. Fenzellii is enlightening (fig. 10). The four vessels shown were four adjacent elements in the same radial row; and from the variation in the vessel perforations within this small number of chances, we are given some indication as to the rôle ontogenetic forces must play in the determination of the end product. Nevertheless, the tendency to produce the scalariform type of vessel perforation is present.

The rays of certain species, being heterogeneous IIA, are less highly organized than those of the remaining genera. Thus, Engelhardtia, as a genus, although on an average more specialized anatomically than Alfaroa, retains many primitive features. This fact parallels the conclusions of Manning.

Kribs comments on the fact that, on anatomical grounds, *Pterocarya* resembles the soft-wooded species of *Juglans*. The present investigation confirms this to a degree that for the present purposes the two genera may be treated as a unit. Preliminary studies of the primary wood of the two genera reveal that this anatomical similarity also holds when those structures are compared.

These two genera exhibit tendencies toward ring-porosity, there usually being a gradient in vessel diameter from the early to late wood. However, a few species of Juglans are definitely ring-porous. The vessels of Pterocarya and of a few species of Juglans are fairly thin-walled and retain their angularity; the fiber-tracheids of these species also possess relatively thin walls. The majority of the species of Juglans, however, tend to have vessels and fibers with slightly thickened walls and vessels which are less angular in outline. Inasmuch as the vessels of both genera show only porous perforations, they are more advanced than those of Engelhardtia and Alfaroa.

The ray type of the two genera is relatively constant, so much so that they can be easily distinguished from those of the other genera. In some species of *Juglans* the rays approximate homogeneity, almost reaching the extreme in specialization for this character.

Manning states that the inflorescences of these two genera are closely related as to type and that those of both genera are advanced. However, he asserts that in both male and female catkins Juglans shows a more specialized condition; also he indicates that the stigmas of these genera are of the same

type. This is in general agreement with the anatomical conditions indicated for the two genera.

Carya shows an abundance of specialized characters-a distinct ring-porosity and rounded, thickwalled vessels with only porous perforations distinguish the genus from the previous genera. The rays are of the same general type as those of Pterocarya and Juglans, though they tend to be wider.

According to Manning, Carya, on the whole, possesses the most specialized condition of the inflorescence in the family. The stigmas are advanced as well, and are distinct from the other types which occur in the family. Here again we find that these facts parallel the anatomical specialization of the genus.

Platycarya, as was previously stated, possesses inflorescences of the primitive type. It is unique in the family in that the bracts of the female portion of the inflorescence are conspicuous and woody. Nevertheless, the wood is ring-porous, the vessels, particularly the large ones, having rounded outlines, slightly thickened walls, and only porous perforations. The small vessels and vascular tracheids with which they are associated possess spiral thickenings. According to Frost (1931), such spiral thickenings give evidence of specialization. The rays are apart from the rays of the other genera in that they are the widest and possess the greatest volume.

Platycarya has a stigma similar to that of Pterocarya and Juglans. But on the basis of xylem anatomy it cannot possibly be allied with these genera, nor can it be considered primitive. Disregarding the situation presented by Platycarya, the results of this investigation closely parallel those of Manning's inflorescence and floristic studies. To explain the anomaly presented by Platycarya, one is forced into the realm of theory. Either the evolution of the inflorescence lagged behind that of the xylem anatomy, or there was an early genetic as well as geographic segregation of the genus, or both. This does not solve the problem but does suggest directions in which further investigation may prove fruitful.

Bailey and Tupper (1918) found that as specialization proceeds in the secondary xylem of the dicotyledons, the fusiform cambial initials become shorter and shorter. Because vessels undergo little if any elongation in ontogeny, their lengths should reflect the approximate lengths of the cambial ini-

tials from which they were derived.

From table 1 it is seen that Alfaroa, with the most primitive vessels, has the longest vessel members. Engelhardtia, the other genus with scalariform vessel perforations, comes next in descending order of vessel member length. Pterocarya is less specialized than Juglans, and it also has longer vessels. Carya, with extreme ring-porosity, thick-walled, rounded vessels with porous perforations, shows a decided shortening in vessel length. Shortest of all are the vessels of Platycarya, and this serves to emphasize the anatomical evidence for specialization in the genus.

The additional figures of the table concerning the vessel members are in line with the evidence for specialization derived from the lengths of these members. Bailey and Tupper (1918), and also Frost (1930a, 1930b), state that accompanying the decrease in length of the vessel members, the diameter increases and the angle of the end wall approaches 90°. Engelhardtia and Pterocarya show little difference in the values for the vessel diameters and for the angles of the end walls of the vessel members. One who works with sections of xylem recognizes that real difficulties exist in obtaining adequate and accurate measurements of vessel diameters and endwall angles from sectioned material. In consequence he utilizes the figures obtained to indicate trends and makes no pretense of having results which can be statistically analyzed. Differences of the order of those existing between Engelhardtia and Pterocarya are insignificant. Especially is this true when dimensions vary with the place of origin of the wood in the stem. The most that can be said is that on the basis of length of vessel segment, Engelhardtia shows less specialization than Pterocarya. On the other hand, though Pterocarya and Juglans are much alike structurally and in the organization of their inflorescences (Manning, 1938a), there are significant differences in the figures provided in table 1. Pterocarya seems distinctly less specialized.

The averages for the lengths of the fibers do not afford data which seem significant in the present connection. As Bailey and Tupper (1918) have indicated, the lengths of the fibrous elements are dependent on (1) the lengths of the cambial initials from which they are derived, and (2) the amount of elongation which they undergo. With the possibility of various internal conditions influencing the lengths of these elements, a greater amount of variation

might be expected.

Thus it is evident that, to a significant degree, the results of Manning's study of the inflorescences of

TABLE 1.

	Vessel member length in $\mu$	$\begin{array}{c} \text{Vessel} \\ \text{diameter} \\ \text{in } \mu \end{array}$	Angle end wall of vessel member	Fiber length in $\mu$
Alfaroa	901	100	45°	1,343
Engelhardtia		146	48°	1,481
Pterocarya		140	470	1,339
Juglans		191	58°	1,503
Carya		246	69°	1,516
Platycarya		193	65°	1,479

the Juglandaceae on the one hand and those of the present investigation of the wood anatomy of this family on the other are in accord with one another. This fact offers support to the opinion that continued attempts to approach a natural classification of the Angiosperms must depend upon the cooperative enterprise of individuals from the various fields of botanical endeavor.

It should be added that, within those genera having several species-viz., Engelhardtia, Pterocarya, Juglans, and Carya—attempts to find lines of xylem specialization which would accord with the existing taxonomic arrangement of the species within each genus, or with the suggested lines of specialization in the inflorescence of each genus as suggested by Manning (1938a), have failed. This may in part be due to lack of adequate anatomical material for a study of the range of xylem types. It is, however, more probable that the anatomist has not yet acquired adequate criteria for such intrageneric studies. Those criteria employed here have proved significant for cooperative investigations with the taxonomist on problems involving major phyletic lines of families or even subfamilies. Much more information is needed before anatomy can be employed for work within the genus. Moreover, there is at present little evidence to suggest that anatomical specialization must follow hand-in-hand with the specializations of the flower or of the inflorescence. It may well be that such has occurred but there seems no a priori reason to suggest that it must do so.

### SUMMARY

Manning (1938a) has utilized evidence obtained from a study of the inflorescences found in the Juglandaceae for an interpretation of generic relationships within the family. The present investigation was undertaken in an effort to determine to what degree a study of the xylem anatomy might confirm his findings. There were available for this study wood specimens from a greater majority of the species of the family. The criteria employed were those now generally recognized as significant in phylogenetic studies based on secondary xylem. The findings are as follows:

Alfaroa seems to be the most primitive genus of the family. Engelhardtia is not dissimilar but is somewhat more advanced. Pterocarya and Juglans are closely allied with many anatomical features in common, though Juglans has reached a somewhat higher degree of specialization. Carya has attained a level of structural organization not found in any other genus. The isolated genus Platycarya seems to have achieved a high degree of organization along an independent pattern of specialization.

The results of this investigation are in striking accord with those of Manning. *Platycarya* alone demands a different interpretation. Manning finds this genus generalized in inflorescence for the family and suggests it as primitive. Anatomical evidence cannot support this belief.

Intensive study of intrageneric variations in anatomy gives few facts to support the idea that the established criteria employed in phylogenetic investigations based on anatomy are adequately refined for intrageneric interpretations.

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## GROWTH FACTOR REQUIREMENTS OF FOUR SPECIES OF ISOLATED ROOTS 1

James Bonner and Philip S. Devirian

Although the culture of isolated root tips was reported in 1922 by Robbins and by Kotte, it was not until more than ten years later that White (1934) succeeded in maintaining excised tomato roots in vitro with undiminished growth rate over long periods of time. White found that for the successful culture of tomato roots the addition of a small amount of yeast extract to the nutrient medium was essential. Much of the further work on the cultivation of isolated roots has centered around the identification of the chemical substances responsible for this promotive effect of yeast extract on root growth. Bonner (1937) showed that vitamin B<sub>1</sub>, a component of yeast extract, is essential for the growth of the isolated pea root. A similar relation of vitamin B<sub>1</sub> to the growth of the isolated tomato root has been demonstrated by Robbins and Bartley (1937) and later by White (1937). It has been recognized, however, that vitamin B<sub>1</sub> is not the sole accessory root growth substance supplied by yeast extract. For the pea root nicotinic acid is a second essential factor (Addicott and Bonner, 1938). For the tomato root, on the other hand, vitamin B6 has been shown to be indispensable (Robbins and Schmidt, 1939a, 1939b). In the past, work concerning the root growth factors has centered on the roots of pea and tomato. In the present paper a comparison will be made between the growth factor requirements of the roots of four species of plants.

MATERIALS AND METHODS.—In all the cultures reported here the nutrient solution reported earlier (Bonner and Addicott, 1937) as satisfactory for the growth of isolated pea roots was used. This nutrient solution contains per liter: 242 mg. of Ca(NO<sub>3</sub>)<sub>2</sub>·4H<sub>2</sub>O, 42 mg. of MgSO<sub>4</sub>·7H<sub>2</sub>O, 85 mg. of KNO<sub>3</sub>, 61 mg. of KCl, 20 mg. of KH<sub>2</sub>PO<sub>4</sub>, and 1.5 mg. of ferric tartrate. Sucrose was added to the medium in a final concentration of 4 per cent for roots of pea, radish and flax, and of 2 per cent for tomato roots.

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Accessory growth factors were made up in concentrated stock solutions so that they could be added, as desired, at the rate of 1.0 cc. of stock solution per liter of nutrient. The final medium was autoclaved at 15 pounds pressure for 25 minutes.

All cultures were carried out in 10 cm. Petri dishes, each dish containing 20 cc. of nutrient solution and 1 to several roots, the number of roots depending inversely on the luxuriance of growth.

Culturing and transferring of the roots were carried out in a culture room which could be steam sterilized. Forceps and knives were sterilized by immersion in alcohol and subsequent flaming. Contaminations by fungi or bacteria were very rare. All cultures were maintained in the dark in an air-conditioned room held at 25 ± 0.5°C.

Roots were in all cases obtained from seedling plants. Seeds were sterilized in 0.1 per cent HgCl<sub>2</sub> and allowed to germinate under asceptic conditions. When the seedling roots were 10-20 mm. long, tips 4-5 mm. long were excised and used for further work.

EXPERIMENTAL RESULTS .- Pea roots .- It is known (Addicott and Bonner, 1938) that isolated pea roots can be maintained in culture over periods of several months (and presumably indefinitely) provided that both vitamin B<sub>1</sub> and nicotinic acid are supplied as growth factors in addition to the nutrient medium described above. In the presence of adequate supplies of vitamin B<sub>1</sub> and of nicotinic acid still other factors, however, must limit the growth of the pea root. The relation of other known or possible plant growth substances to the growth of isolated pea roots in the presence of an excess of vitamin B<sub>1</sub> and nicotinic acid was therefore investigated. In each experiment 500 freshly excised tips (variety "Perfection") were grown for one week in growth factor free medium. At the end of this week 400 roots, as nearly uniform as possible, were subcultured by the removal of 10 mm. tips and divided among the several parts of the experiment. The roots were then subcultured at regular weekly intervals. Table 1 gives the results from one experiment in which ade-

Table 1. Neither adenine nor vitamin  $B_6$  exerts any significant effect on the growth of isolated pea roots. Approximately 100 roots in each part.

		Gı	owth in	mm. per 2-week		eraged (	over	
				Weeks in	ı culture	:		
Growth factor added to medium	2	and 3	4 and 5	6 and 7	8 and 9	10 and	11	12
Vitamin B <sub>1</sub> .1 mg./l. + nicotinic acid 0.5 mg	./1	50	63	66	73	84		85
As 1, plus adenine 0.1 mg./l		52	53	67	78	86		87
As 1, plus vitamin B <sub>6</sub> , 0.1 mg./l		53	63	59	76	87		80
As 1, plus adenine 0.1 mg./l. + vitamin B			t et ge					
		50	58	66	71	77		79

Table 2. Vitamins E, K, and B<sub>2</sub> are without effect on the growth of isolated pea roots.

Approximately 100 roots in each part.

	Growth in mm. per week, averaged over 2-week periods Weeks in culture					periods
Growth factors added to medium	2 and 3	4 and 5	6 and 7	8 and 9	10 and 11	12 and 13
Vitamin B <sub>1</sub> 0.1 mg./l. + nicotini	3					
acid 0.5 mg./l		61	64	79	83	73
As 1, + vitamin K conc. 10 mg./l	. 59	60	76	85	83	71
As 1, + vitamin E 1 mg./l	. 55	60	58	82		'a a a <b></b>
As 1, + vitamin $B_2$ 1 mg./l		67	64	88	85	72

nine (a factor for the growth of leaves, D. Bonner and Haagen-Smit, 1939) and vitamin B62 were used as additions to vitamin B1 and nicotinic acid. Concentrations of adenine above 0.5 mg. per liter were found to be inhibitory and 0.1 mg. per liter was therefore used. Vitamin B6 was used at the concentration of 0.1 mg. per liter, a concentration satisfactory for the maintenance of isolated tomato roots. (See below.) It is evident from table 1, however, that neither of these known plant growth factors exerted any significant effect on the growth of pea roots over the 12-week experimental period. In table 2 are presented results from a similar experiment with vitamins E, K, and B2.2 Vitamin E was given as pure tocopherol, vitamin K as a purified concentrate, and vitamin B2 as crystalline riboflavin. As may be seen in table 2, none of these substances exerted a significant effect on the growth of pea roots over the 14-week experimental period. These substances were also used in concentrations 10 or 100 times lower than those given in table 2 without significantly different results. Pantothenic acid, β-alanine,2 ascorbic acid, and theelin were likewise without effect over wide ranges of concentration. Addicott (1939) has reported 20 amino acids as being without significant growth factor effect on the growth of pea roots in the presence of vitamin B1 and nicotinic acid.

It may then be concluded that the growth of pea roots in the presence of vitamin  $B_1$  and nicotinic acid is not limited by any of the above substances and growth factors. It is of particular interest that adenine, known to be a growth factor for leaves, and vitamin  $B_6$ , known to be a growth factor for tomato roots, are neither essential nor promotive to the growth of isolated pea roots under the present experimental conditions.

Radish roots.—Two hundred and fifty tips from the roots of asceptic radish seedlings (variety "Scarlet Globe") were excised and cultured in media of the compositions shown in table 3. Two transfers were made at weekly intervals and two further transfers at two weekly intervals. At the end of this time a parallel series of cultures in 2 per cent sucrose me-

<sup>2</sup> We are indebted to Prof. Samuel Lepkovsky, Berkeley, for the crystalline vitamin  $B_6$ ; to Dr. R. T. Major, Merck and Co., Inc., for the supply of vitamins E, K, and  $B_2$ ; and to Prof. R. J. Williams, Corvallis, for the pantothenic acid and the β-alanine.

Table 3. The growth factor requirements of excised radish roots. Mineral nutrient of basic pea root medium and 4 per cent sucrose used in all cases.

Added growth factors	Observations
None	Ceased growth after 4th transfer
Vitamin B <sub>1</sub> 0.1 mg./l	Ceased growth after 4th transfer
Nicotinic acid 0.5 mg./l.	Growth slight after 4th transfer
$B_1$ + nicotinic acid	Growth constant at approxi- mately 30 mm. per 2 weeks for 14 transfers
Yeast extract 0.01%	Growth identical with that in $B_1 + nicotinic$ acid medium

<sup>a</sup> The yeast extract was made by boiling 1 gram of dried brewer's yeast in 100 cc. of water for 30 minutes, filtering, and making up to 100 cc. One cc. of this extract, representing the water soluble portion of 10 mgs. of dried yeast, diluted to 100 cc. is referred to as 0.01 per cent yeast extract.

dium had ceased their growth. Of those in 4 per cent sucrose medium, the control (which received no accessory growth substances), and the vitamin B<sub>1</sub> cultures also showed no new growth. The cultures which received nicotinic acid grew only to a limited extent. However, if yeast extract or vitamin B<sub>1</sub> and nicotinic acid together were present in the medium, growth of the isolated radish roots was vigorous, as is shown in figure 1. No significant differences in luxuriance of root growth between yeast extract and vitamin B<sub>1</sub>-nicotinic acid cultures could be detected, and it would therefore appear that this combination of pure crystalline growth factors is capable of completely replacing yeast extract for the radish root. The roots of the vitamin B<sub>1</sub>-nicotinic acid series were increased in number by propagation from secondary root tips and were kept in culture through 14 transfers over a period of 6 months. During this time the average growth rate was maintained at approximately 30 mm. per root (as judged by the longest root produced by each fragment) per two weeks. Since this growth rate is slow compared to that of pea or tomato roots, numerous attempts to increase it were made. Additions of vitamins E, K, and B2, pantothenic acid, adenine, the known minor elements, or of the amino acids asparagin, glutamic acid, glycine, isoleucine, leucine, tryptophane, and valine were all without influence on the growth rate. Vitamin B<sub>6</sub> also was completely without effect. White (1938) has reported the culture of excised radish roots in yeast extract medium through several passages at an average growth rate of 13.5 mm. per day. White does not give the variety with which he worked, but it seems probable that it may have differed from that used in the present work. In any event, it is possible to culture radish roots of the present variety through 14 passages (and hence presumably indefinitely) in a medium containing only vitamin B<sub>1</sub> and nicotinic acid as accessory growth factors. It might be noted that no "radishes" were formed in any of these cultures of isolated roots, and none would be expected, since the "radish" is largely a hypocotylar structure in the globe types (Havward, 1938).

Flax roots.—Approximately 500 root tips were removed from asceptic flax seedlings (unnamed variety) and maintained for one week in the standard pea root medium with 4 per cent sucrose. At the end of this week, during which the roots grew an average of approximately 150 mm., they were transferred to media either with or without vitamin B1. The roots were then subcultured weekly into fresh media of the same composition. The growth rate of the roots which received no vitamin B<sub>1</sub> declined steadily. Those roots which received B1, however, grew luxuriantly and maintained a growth rate of more than 2 cm. per day through 14 weekly transfers. The addition of either nicotinic acid or vitamin B6 to the medium in which flax roots were cultured resulted in no further increase of growth rate (table 4). Vita-

Table 4. Growth of isolated flax roots in media of different composition. Mineral nutrient of basic pea root medium and 4 per cent sucrose was used in all cases.

Added growth factors	Observations
None	Growth declined to 67 mm. per week after 5 weeks
Vitamin B <sub>1</sub>	Growth maintained at an average of 158 mm. per week
Nicotinic acid	Growth declined to 60 mm. per week after 5 weeks
Vitamin B <sub>6</sub>	Growth declined to 61 mm. per week after 7 weeks
$B_1 + nicotinic acid$	Growth maintained at an average of 165 mm. per week
$B_1 + B_6 \dots$	Growth maintained at an average of 153 mm. per week
Nicotinic acid $+ B_6 \dots$	Growth declined to 43 mm. per week after 7 weeks
${\bf B_1} + { m nicotinic} \ { m acid} + {\bf B_6}$	Growth maintained at an average of 140 mm. per week.

min  $B_1$  is apparently the only necessary growth factor required for the luxuriant growth in vitro of isolated flax roots.

Tomato roots.—Tips from two hundred asceptic tomato seedlings (variety "Beefsteak") were cultured in medium containing 2 per cent sucrose and

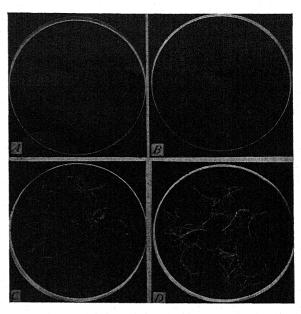


Fig. 1. Growth of isolated radish roots in different media. Photographed 2 weeks after the 4th subculturing. Approximately the same number of initial root tips was used in each case. A, basic media + vitamin B<sub>1</sub>, 0.1 mg./l.; B, basic medium + nicotinic acid, 0.5 mg./l.; C, basic medium + 0.01 per cent yeast extract; D, basic medium + vitamin B<sub>1</sub>, 0.1 mg./l. + nicotinic acid, 0.5 mg./l.

0.01 per cent yeast extract in addition to the standard mineral nutrient. At the end of one week, the 10 most vigorous roots were selected and a clone established from each. One of these 10 clones exhibited a considerably greater luxuriance of growth than did the other 9 and was hence selected for all the work reported below. Roots from this clone were maintained through 12 weekly passages on a medium containing only vitamin B<sub>1</sub> and nicotinic acid as acces-

Table 5. Growth of excised tomato roots in media of different composition. Mineral nutrient of basic pea root medium and 2 per cent sucrose used in all cases.

Added growth factors	Observations
None	Growth ceased after 2nd trans- fer
Vitamin B <sub>1</sub>	Growth ceased after 2nd transfer
Nicotinic acid	Growth ceased after 2nd trans- fer
Vitamin B <sub>6</sub>	Growth ceased after 3rd trans- fer
B <sub>1</sub> + nicotinic acid	Growth slow (approximately 10 mm. per week), discarded after 3 weeks
Nicotinic acid $+ B_6$	Growth ceased after 3rd trans- fer
$B_1 + B_6 \dots$	Growth maintained at 40 mm. per week for 18 weeks
$B_1 + nicotinic acid + B_6$	Growth maintained at 60 mm. per week for 20 weeks

sory growth factors. Growth was maintained at approximately 10 mm. per week. At the end of this time, the roots were subcultured into the media shown in table 5. Vitamin B<sub>6</sub> in combination with vitamin B1 exerted a striking promotive effect on the growth of these isolated tomato roots, a finding in agreement with and corroborating that of Robbins and Schmidt (1939a). Roots cultured in medium containing these two growth factors grew at an average rate of 40 mm. per week. Table 5 shows in addition that this growth rate may be increased to approximately 60 mm. per week through the addition of nicotinic acid to the medium. Neither vitamin B1, nicotinic acid, nor vitamin B6 alone was able to support growth at any appreciable rate. It would seem justifiable to conclude, therefore, that this clone of tomato roots is heterotrophic for vitamin B1 and vitamin B<sub>6</sub>, and to some extent also for nicotinic

It has been reported (Robbins and Bartley, 1937) that the vitamin B<sub>1</sub> thiazole (4 methyl, 5-\beta-hydroxyethyl thiazole) is capable of replacing vitamin B1 in the nutrition of isolated tomato roots. During the course of the present investigation a few observations on the growth of tomato roots in thiazole solutions were made. These are summarized in table 6. Roots of the present clone grown in medium containing only vitamin thiazole as an accessory growth factor completely ceased their growth after two transfers. Roots grown in medium containing vitamin B6 in addition to the vitamin thiazole decreased steadily from an initial growth rate of 45 mm. per week to 0 mm. per week after nine weeks. If nicotinic acid was also present in the medium, growth declined from an initial rate of approximately 65 mm.

Table 6. Effect on the growth of isolated tomato roots, of replacing vitamin B<sub>1</sub> by vitamin thiazole. Mineral nutrient of basic pea root medium and 2 per cent sucrose used in all cases.

Added growth factors Observations		
Vitamin thiazole	Growth ceased after 2 weekly transfers	
Vitamin thiazole + vita-		
min B <sub>6</sub>	Growth declined to 0 during 9 weekly transfers	
Vitamin thiazole + vitamin B <sub>6</sub> + nicotinic		
acid	Growth declined to 8 mm. per week during 9 weekly trans- fers	
Vitamin B <sub>1</sub> + nicotinic		
acid $+$ vitamin $B_6$	Growth maintained at 60 mm.	

per week to 8 mm. per week after nine weeks. At the end of this time the roots were short and slender in comparison to the parallel series of roots which received vitamin  $B_1$  rather than vitamin thiazole, and were discarded. Tomato roots of the present clone can therefore not thrive when supplied with vitamin thiazole in place of vitamin  $B_1$ , and it may be con-

per week

cluded that they synthesize little if any vitamin pyrimidine. This conclusion is supported by direct assays for vitamin  $B_1$  made with the *Phycomyces* test (Schopfer and Jung, 1937; Bonner and Erickson, 1938) on tomato roots cultured for five weeks with nicotinic acid and vitamin  $B_6$  plus either vitamin  $B_1$  or vitamin thiazole. The roots supplied with vitamin  $B_1$  contained more than ten times as much of this substance as roots cultured with vitamin thiazole.

Discussion.—The isolated roots of the four species of plants reported here differ as to their accessory growth factor requirements. Thus, isolated roots of flax require vitamin  $B_1$  alone, roots of radish and pea require vitamin  $B_1$  and nicotinic acid, and roots of tomato require vitamin  $B_1$ , nicotinic acid, and vitamin  $B_6$  for maximum rate of growth. It might be suspected that vitamin  $B_6$  is essential to the growth of pea, flax, and radish roots and that nicotinic acid also is required by roots of flax but that these requirements are met by in vivo synthesis of the substances in the several cases. The nature of such possible in vivo synthesis of accessory growth factors by isolated roots, however, will be the subject of future communications.

### SUMMARY

Isolated pea roots can be cultivated indefinitely in nutrient medium containing vitamin  $B_1$  and nicotinic acid in addition to mineral salts and 4 per cent sucrose. The growth rate in this medium is 70–85 mm. per week. Vitamin  $B_6$ , adenine, ascorbic acid, theelin,  $\beta$ -alanine, pantothenic acid, vitamins E, K, and  $B_2$ , and numerous amino acids are without effect in increasing the growth rate of isolated pea roots in the presence of vitamin  $B_1$  and nicotinic acid.

Isolated radish roots were cultivated through 14 passages over a period of six months at an average growth rate of 30 mm. per two weeks in medium containing vitamin  $B_1$  and nicotinic acid in addition to the basic nutrient. Both vitamin  $B_1$  and nicotinic acid were essential for the maintenance of growth. Vitamins  $B_6$ , E, K, and  $B_2$ , adenine, pantothenic acid, and numerous amino acids were without influence on the growth rate.

Isolated flax roots were cultivated through 14 weekly transfers at an average growth rate of approximately 150 mm. per week. Vitamin  $B_1$  is the only accessory growth factor required by the present strain of flax roots. Additions of vitamin  $B_6$  or nicotinic acid were without significant effect on the growth rate.

In confirmation of earlier work by others (Robbins and Schmidt, 1939a), it is shown that isolated tomato roots can be cultivated indefinitely at an average growth rate of 40 mm. per week in medium containing only vitamins  $B_1$  and  $B_6$ . The growth rate, however, could be increased to 60 mm. per week by the addition of nicotinic acid to the medium.

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# THE PATH OF FLUORESCEIN MOVEMENT IN THE KIDNEY BEAN, PHASEOLUS VULGARIS <sup>1</sup>

## Edward M. Palmquist

The technique developed by Schumacher (1933) for following visually the path of a non-toxic solute as it moved in plants extended the possible scope of experiments dealing with translocation. Using this technique, in an attempt to determine whether or not two different solutes could move simultaneously in opposite directions in the same phloem tissue, the writer (1938) corroborated Schumacher's finding that fluorescein moved in the phloem if proper precautions were taken. The evidence for this was merely the detection of the greenish yellow fluorescence, characteristic of fluorescein, in the phloem of the petioles of bean leaves to which fluorescein had been applied. This fluorescence was absent in the petioles of untreated leaves.

It was also found that under certain conditions fluorescein can be made to move more rapidly in the xylem than in the phloem. When the cut end of a stem was immersed in fluorescein solution, for example, the solute rose rapidly in the xylem. Likewise, when a scraped leaf of a water deficient plant was immersed in fluorescein solution the solute moved rapidly out of the leaf through the xylem. It also moved rapidly in the xylem from an immersed tip of a potato sprout toward the shrivelling tuber. In each of these instances in which movement occurred in the xylem, there was undoubtedly a corresponding mass movement of water. In the cut stems, fluorescein moved with the transpiration stream, and in the other two illustrations there was a mass movement of water in the xylem from the immersed portion toward parts of the plant which were deficient in water. It would indeed be hard to visualize a mecha-

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nism in plants which would prevent the movement of fluorescein, or any other solute, with the transpiration stream, once it had entered the xylem. Fluorescein cannot be carried along in this manner in well watered or turgid plants which exhibit little or no mass movement of water.

Rhodes (1937) has added a new line of evidence from which he concludes that fluorescein moves in the xylem and that the apparent movement in the phloem is merely the result of lateral diffusion into that tissue following the movement in the adjacent xylem. He found, as did Schumacher, that after the application of fluorescein to a leaf, sections of the stem below the treated leaf showed the characteristic vellow fluorescence in the phloem and not in the xylem. He pointed out, however, that fluorescein might be present in the xylem in a non-fluorescent state, since it does not fluoresce strongly in acid solution, and the sap of most plants is acid. He therefore exposed his sections to ammonia vapor before examination and found that in sections of the stem immediately below a fluorescein treated leaf, both xylem and phloem showed the characteristic yellow fluorescence, and further, that as sections were taken progressively farther away from the treated leaf, fluorescence was present in the xylem below the point where it faded out in the phloem. This evidence at first sight seems to indicate strongly that either fluorescein moves more rapidly in the xylem than it does in the phloem, or it moves only in the xylem and subsequently diffuses laterally into the adjacent phloem.

Schumacher (1937), however, using fluoresceintreated plants of *Pelargonium*, found that ammonia

TARLE 1.

	Sections untreated	Sections exposed to ammonia vapor	Sections exposed to HCl vapor
Acanthopanax	Greyish yellow	Bright yellow	None
Acer	Greyish yellow	Bright yellow	None
Ampelopsis	Strong yellow	Brilliant greenish yellow	None
Berberis	Brilliant yellow	Brilliant yellow	Brilliant yellow
Trataegus	Faint yellow	Yellow	None
Ligustrum	Faint yellow	Bright yellow	None
Lonicera	Faint yellow	Bright yellow	None
Maclura	Dull orange	Bright yellow	None
Philadelphus	Faint yellow	Bright yellow	None
Populus	Faint yellow	Bright yellow	None
Prunus	Faint yellow	Bright yellow	None
Quercus	Faint yellow	Bright yellow	None
Rhamnus	Faint yellow	Yellow	None
Rhus	Dull orange	Bright yellowish green	None
Robinia	Pale orange yellow	Brilliant yellow	None
Rosa	Dull yellow	Yellow	None
Sambucus	Dull yellow	Bright yellow	None
Spiraea	Dull orange	Yellow	None
Symphoricarpos	Yellow	Bright yellow	None
Syringa	Grevish yellow	Bright yellow	None
l'axus	Greyish yellow	Yellow	None
Tilia	Greyish yellow	Yellow	None
Ulmus	Pale yellow	Brilliant yellow	None
Vitis	Dull orange-yellow	Bright greenish yellow	None

vapor treatment did not bring about fluorescence in the xylem. He points out that Rhodes did not follow his gelatin block method of applying fluorescein but instead immersed leaves in aqueous solutions. He assumes that with this method Rhodes probably introduced fluorescein into the xylem where it could move with the transpiration stream.

The writer (1938) has pointed out that many plants possess inherent fluorescent compounds, some of which, under the stimulation of blue and violet light, emit a yellowish fluorescence practically identical to that of fluorescein. Many untreated woody plants were examined under the fluorescence microscope, and without exception the xylem showed some yellowish florescence, although the intensity of this fluorescence varied greatly. The effect of ammonia vapor on the fluorescence of these woods was ascertained. Cross sections of twigs were exposed to ammonia vapor for three minutes before examining. In all the twigs so treated the xylem fluoresced with a brilliant yellow light. These plants had not been treated with fluorescein. With one exception (Berberis) the same sections when exposed to hydrochloric acid fumes lost this fluorescence, but regained it after a second exposure to ammonia vapor. A list of some of the genera examined and a description of the natural fluorescence in the xylem are given in table 1.

It is apparent from these data that the selection of material is a most critical task in the preparation of experiments with fluorescein. The use of plants which show natural fluorescence, similar to that of fluorescein, whether treated with ammonia vapor or not can lead only to confusion. Of all the plants examined, the common red kidney bean (Phaseolus vulgaris L.) has proved most satisfactory. Sections taken from the young parts of this plant show no fluorescence in the xylem, either with or without the ammonia treatment. In the old parts of this plant. however, and also in those of *Pelargonium*, the plant used originally by Schumacher, the xylem shows a yellow fluorescence when treated with ammonia vapor. In addition to the careful selection of material, it is essential that mass movement of water in the xylem be prevented or at least recognized. It should be obvious that a cut stem, exposed to conditions which allow transpiration, with its base immersed in fluorescein solution will rapidly take up the solution in its xylem and that an intact but water deficient plant will do likewise if a leaf or stem tip is immersed in fluorescein solution.

THE PATH OF FLUORESCEIN MOVEMENT IN BEAN PLANTS.—Last July, twenty young potted bean plants were moved from the greenhouse to a dark room and watered thoroughly. The terminal leaflet from one compound leaf on each plant was gently scraped and immersed in a one-tenth per cent fluorescein solution. A short section of the rachis was scalded on ten leaves. Three hours later one lateral leaflet was removed from each of the treated leaves and examined macroscopically for fluorescence. The leaflets from the leaves with scalded rachises showed no fluorescence, while those from leaves the rachises of which had not been scalded showed brilliant fluorescence along the veins. Twenty-one hours later the second lateral leaflet was removed from each of the twenty leaves. The results here were identical with those observed after three hours, except that at this time two of the ten leaflets from the scalded leaves showed fluorescence along the veins.

Cross sections of the stems below the treated leaves were examined under the fluorescence microscope. Those taken from plants with scalded rachises showed no fluorescein in either xylem or phloem, while those from plants with unscalded rachises were brilliantly fluorescent in the phloem. Similar sections were exposed to ammonia vapor for three minutes. This treatment did not bring about any change in the fluorescence of the conducting tissues. No fluorescein could be detected in the xylem of any of the plants.

From these facts it was concluded that under conditions which prevent a mass movement of water in the xylem, the tissue that conducts fluorescein in bean plants is the phloem.

### SUMMARY

Schumacher reported that fluorescein moves in the phloem. Later, Rhodes concluded that it moves in the xylem, but ordinarily is not detectable there without raising the pH, and that the apparent movement in the phloem results from the lateral diffusion into that tissue from the adjacent xylem. Cross sections of untreated twigs, representing twenty-five genera, were examined with a fluorescence microscope. Most of these showed a trace of yellow fluo-

rescence in the xylem. These sections were then exposed to ammonia vapor. The xylem of all of them fluoresced with a brilliant yellow light similar to that of fluorescein. These twigs had not been treated with fluorescein. The xylem of young bean plants does not fluoresce, either with or without the ammonia treatment. Fluorescein was applied to a series of thoroughly watered bean plants. Twenty-four hours later, cross sections of the stems showed fluorescence in the phloem, but not in the xylem. Exposure to ammonia vapor failed to produce fluorescence in the xylem. It is concluded that fluorescein moves in the phloem of bean plants.

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## A SECOND GROWTH FACTOR FOR EXCISED PEA ROOTS: NICOTINIC ACID <sup>1</sup>

Frederick T. Addicott and Philip S. Devirian

WHITE (1934) has shown that it is possible to cultivate excised tomato roots indefinitely under sterile conditions. The medium which he used contained yeast extract in addition to salts and sugar. Subsequent workers have directed their attention to the determination and study of the substances in yeast extract which are necessary for the growth of roots. Bonner (1937), working with pea roots, and Robbins and Bartley (1937), working with tomato roots, simultaneously and independently showed that vitamin B<sub>1</sub> was an essential component of the yeast extract in which roots of both these plants had previously been grown. However, it soon became evident that vitamin B<sub>1</sub> could not completely replace yeast extract; when supplied as the only accessory substance, it would not support the indefinite growth of pea roots. This paper reports the results of a search for the other growth factor(s) of excised pea roots. A preliminary note on this work was recently published (Addicott and Bonner, 1938).

Метнорs.—The methods used in this work have already been discussed in detail (Bonner and Addi-

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cott, 1937); so they need only be mentioned briefly here. Seeds of garden peas (Pisum sativum) of the variety Perfection were germinated under sterile conditions. At the end of two days 5 mm. of the root tip was removed and placed in a 10 cm. Petri dish containing 20 ml. of liquid culture medium. At the end of each succeeding week 10 mm. of the root tip was removed and transferred to fresh medium. The process of weekly subculture was repeated for the duration of each experiment. All the cultures were made under aseptic conditions. The following salts were used in the basic medium:  $(Ca(NO_3)_2 \cdot 4H_20$ , 236 mg.; MgSO<sub>4</sub>·7H<sub>2</sub>O, 36 mg.; KNO<sub>3</sub>, 81 mg.; KCl, 65 mg.; KH<sub>2</sub>PO<sub>4</sub>, 20 mg.; Fe-tartrate, 1.5 mg.; all in 1 liter of double distilled water. The medium also included 4 per cent sucrose as a source of carbohydrate. The substances under consideration were tested for their ability to increase root growth in length during the second weekly transfer, always in the presence of vitamin B<sub>1</sub> (0.1 mg. per liter). Twenty or more roots were used to test the effects of each concentration. If a substance showed signs of activity under these conditions tests were continued through a series of subcultures.

OBSERVATIONS.—It had been suggested in the literature that the second growth factor in the case of tomato roots might be among the microelements (Robbins, White, McClary, and Bartley, 1936; Robbins and Schmidt, 1938) or among the amino acids of yeast extract (White, 1937). The preliminary experiments of Bonner and Addicott (1937) also indicated that the amino acids might be a source of growth factors for pea roots. Both these types of substances were investigated.

The ash of pea seeds was first utilized as a source of microelements, since it is well known that the seed is capable of supplying all the nutritional requirements of a young plant for some time. However, this was found to be completely inactive (table 1).

Table 1. Effect of pea ash on the growth of excised pea roots.

		Growth			
Concentration of ash mg. per liter		Expressed as percentage of roots receiving only standar salts, sucrose, and vitamin I			
	25	71			
	5	77			
	1	82			
	0.1	87			
	0.01	99			
	0.001	105			
	0.0001	100			

Higher concentrations of the ash inhibited growth, and as dilution progressed, growth approached that of the controls. White's (1938) supplementary salt solution was also tested, alone and in combination with an arbitrary mixture of salts. The latter included additional elements known to occur in the Leguminosae and other plants (table 2, 3). With

Table 2. Supplementary salt solutions.

	Salt	Concentration mg. per liter
Solution A	ZnSO <sub>4</sub>	30.0
(after White, slightly modified)	Na <sub>2</sub> SiO <sub>3</sub>	27.0
물림 회의 지원 등에 보고하는 것이	$Al_2(SO_4)_3$	107.0
	KI	15.0
얼마나 바다 하는 사람이 사이	$H_3BO_3$	32.0
	NaCl	56.0
	MnSO <sub>4</sub>	44.0
	NiCl <sub>2</sub>	4.0
	LiCl	3.9
	$CoCl_2$	4.0
	$Cu(NO_3)_2$	1.0
Solution B	TiO <sub>2</sub>	100.0
	SrCl <sub>2</sub> .6H <sub>2</sub> O	100.0
	$Ba(CO_3)_2$	100.0
	Na3AsO4.12H2O	100.0
	$CrO_3$	100.0
	Na <sub>2</sub> WO <sub>4</sub> .2H <sub>2</sub> O	100.0
in adical in granting a	CdCl <sub>2</sub> .2H <sub>2</sub> O	100.0

Table 3. Effect of supplementary salt solutions on the growth of excised pea roots.

Conc. sol'n A ml. per liter	Conc. sol'n B	Growth Expressed as percentage of roots receiving only standard salts, sucrose, and vitamin B <sub>1</sub>
250		4
100		91
50		97
10		85
5		73
1		92
0.1		99
0.01		87
10	1	79
5	1	55
5	0.1	81
••	0.01	85

these, likewise, no dilution or mixture was found in which the roots grew to a greater extent than they would with vitamin  $B_1$  alone in standard salts and sugar. In the hope that these negative results were due to the presence of one or more salts in concentrations inhibitive to growth, some experiments were run using single salts. These were also negative, additional zinc, manganese, copper, and tungsten being without significant effect (table 4). This list would have been extended had it not appeared in experiments which will be discussed shortly that the limiting factor under the above conditions was nicotinic acid and not to be found among the inorganic substances at all.

Table 4. Effect of microelements, given as single salts, on the growth of excised pea roots.

Salt	Concentration mg. per liter	Growth Expressed as percentage of controls not supplied the salt		
CuSO <sub>4</sub> .5H <sub>2</sub> O	1	21		
	0.1	102		
	0.01 0.001	100 106		
MnSO <sub>4</sub>	10	35		
	1	105		
	0.1	99		
	0.01	102		
$Na_2WO_4.2H_2O$	10	68		
	1	94		
	0.1	107		
	0.01	102		
ZnSO <sub>4</sub>	10	78		
	1	84		
	0.1	85		
	0.01	88		

Table 6. Growth of excised pea roots when supplied combinations of vitamin  $B_1$ , amino acids, and nicotinic acid.

	Average per	week in mr	Growth week in mm. ± standard error of the mean			
Accessory factors	2 and 3	4 and 5	Weeks 6 and 7	8 and 9	10	
Vitamin B <sub>1</sub>	. 39.7±0.9	30.4±1.0	10.4±1.0	1.8±0.8	$0.7 \pm 0.4$	
Vitamin B <sub>1</sub> plus amino acids Vitamin B <sub>1</sub> plus amino acids plus nicotinio		38.5±0.9	18.2±2.0	2.5±1.4	$0.9\pm0.9$	
acid		$55.8 \pm 3.5$	$57.4 \pm 4.8$	64.5±1.8	$60.4 \pm 6.3$	

The search for the additional growth factor was next carried to the amino acids. Twenty of the highest purity available were selected for use in a series of experiments. These were: alanine, arginine, asparagine, aspartic acid, cystein, cystine, glutamic acid, glycine, isoleucine, leucine, lysine, methionine, ornithine, phenyl-alanine, proline, serine, tryptophane, tyrosine, and valine. Each amino acid was tested singly and in combination with other acids for its effect on growth during the second weekly transfer. Ten were found to increase root growth significantly over that of the controls receiving only vitamin B<sub>1</sub>. In order to obtain the optimum mixture of amino acids, each of these ten was tested in three concentrations in the presence of the other nine. The results of this experiment are shown in table 5,

Table 5. Effect of ten selected amino acids on the growth of pea roots.<sup>a</sup>

	Concentration			Value of	
Acid	10 X	1 X	0.1 X	1 X in mg. per liter	
Asparagine	97	92	119	1.0	
Glutamic Acid	117	113	90	0.5	
Glycine	132	138	155	0.5	
Histidine	94	94	99	0.15	
Isoleucine	92	112	92	0.015	
Leucine	82	116	112	0.5	
Methionine	68	104	79	0.05	
Serine	70	82	83	0.05	
Tryptophane	79	130	117	0.5	
Valine		115	120	0.05	

<sup>\*</sup> Growth is expressed as the percentage of the length of the control roots which were given all the amino acids (at the concentration of 1 X) except that acid being studied.

showing the optimum concentration of each acid in the presence of the others. It also shows that it was possible to dispense with three of the acids. Remaining were: asparagine, glutamic acid, glycine, isoleucine, leucine, tryptophane, tyrosine, and valine. This combination of amino acids in the concentrations indicated should be optimum for the growth of pea roots.

In order to determine if this mixture could supply the other growth factor(s), it with vitamin  $B_1$  was added to the medium of a series of roots for a period of ten weekly subcultures. Control roots received only vitamin B<sub>1</sub> as an accessory growth substance. In this experiment 250 roots were used. While these cultures were in progress results became available from experiments in which the effects of various other substances, known to be growth factors for either bacteria or fungi, had been tested. From these it appeared that nicotinic acid had a considerable effect on root growth during the second transfer. In order to determine if this would persist in later subcultures, nicotinic acid (0.5 mg. per liter) was added to the medium of part of the roots which had been receiving vitamin B<sub>1</sub> and amino acids in the above experiment. The series was then continued through 10 weekly subcultures in three parts: roots receiving (a) vitamin B<sub>1</sub> alone, (b) vitamin B<sub>1</sub> plus amino acids, (c) vitamin B<sub>1</sub> plus amino acids plus nicotinic acid. The results of this experiment are shown in table 6; and shadow photographs of the

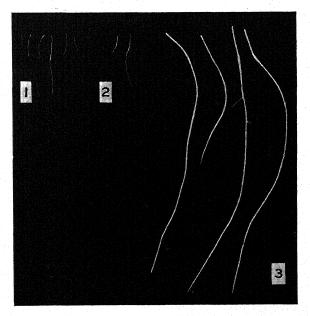


Fig. 1-3. Shadow photographs of roots at the end of the 10th transfer.—Fig. 1. Supplied vitamin  $B_1$  only.—Fig. 2. Supplied vitamin  $B_1$  and amino acids.—Fig. 3. Supplied vitamin  $B_1$ , amino acids, and nicotinic acid. (Five mm. have been removed from the tip of each root.)  $\times 0.8$ .

roots at the end of the tenth transfer appear in figures 1 to 3. From these it is clear that neither vitamin  $B_1$  nor the amino acid mixture with vitamin  $B_1$  is capable of supporting growth indefinitely. However, roots which had received nicotinic acid in addition to the amino acids and vitamin  $B_1$  were growing at a more rapid rate at the end of ten weeks than they were at the start.

Other experiments, which need not be described in detail here, have shown also that nicotinic acid alone could not support root growth for more than a few weeks. It now remained only to be determined whether or not the amino acids were necessary in the successful combination of vitamin B<sub>1</sub>, amino acids, and nicotinic acid found in the above experiment. Consequently a series was started in which part of the roots were placed in medium containing only vitamin B<sub>1</sub> and nicotinic acid as accessory growth factors; the remainder received both these substances and the amino acids in addition. This experiment, started with nearly 500 roots, has at the time of writing extended over 31 weekly subcultures. As the growth rates of figure 4 show, nicotinic acid with

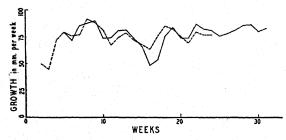


Fig. 4. Weekly growth rate of pea roots over 31 transfers. Solid line: vitamin  $B_1$  and nicotinic acid. Dotted line: amino acids, vitamin  $B_1$ , and nicotinic acid.

vitamin  $B_1$  is completely capable of satisfying the accessory substance requirements of pea roots for indefinite growth. In the presence of these two substances growth (77.1 mm. per week) is even greater than it is in the presence of an optimum concentration of yeast extract (about 60 mm. per week). There was no indication that the amino acid mixture had any effect on the growth rate of the roots when both nicotinic acid and vitamin  $B_1$  were also present in the medium.

Discussion.—It can now be considered as demonstrated that pea roots can grow indefinitely when supplied only vitamin B<sub>1</sub> and nicotinic acid as accessory growth substances. It is of interest to note that the requirements of the roots are so simple. It would not have been surprising if several or many substances of a hormonal nature were required by the isolated roots. The evidence indicates clearly that pea roots when supplied salts, sugar, vitamin B<sub>1</sub>, and nicotinic acid are capable of synthesizing all the proteins, hormones, enzymes, and other substance, both simple and complex, necessary for the formation of living material and for indefinite growth. This growth involves not only increase of

root material but active and continued cell divisions.

It is not surprising that nicotinic acid should be a growth factor for higher plants (its effects on other roots and intact plants are now under investigation in these laboratories), since it is known to be essential to the growth of several microorganisms. Knight (1937) has found it to be necessary for the growth of Staphylococcus aureus. Nicotinic acid is also a growth factor for Staphylococcus albus (Koser, Finkle, Dorfman, Gordon, and Saunders, 1938), for Bacillus proteus (Fildes, 1938), for the dysentery bacillus (Koser, Dorfman, and Saunders, 1938), and for the diphtheria bacillus (Mueller, 1937).

In the bacteria which have been investigated, nicotinic acid amide (the pellagra preventative vitamin) has been found of equal activity with nicotinic acid. In the case of pea roots the preliminary work of Bonner and Buchman (unpublished) with various chemical analogs of nicotinic acid has likewise indicated that the acid amide is as active as the substance itself. This would support the idea that either they are both readily converted by the root to an active form or else that they are both equally active. The first interpretation seems the more likely since nicotinic acid amide is known to be the active part of at least two dehydrogenase systems. The possible action of nicotinic acid through such a system in the root has not yet been investigated.

As shown above, roots cultured in medium containtaining nicotinic acid with vitamin B1 attain in a few weeks a growth rate considerably above the initial rate. Whereas, if yeast extract is given as the source of accessory growth substances, the rate remains at about the initial level. The rise in rate of root growth during the first few weeks in vitamin B<sub>1</sub>-nicotinic acid medium may be due to changes in the auxin content. Van Overbeek (in press) has shown that the root tips at the time of removal from the seed have a high auxin content while as subcultures progress the level falls to a lower point at which it is maintained through production by the root. It has been known that it is possible to inhibit root growth by the application of auxin (Kögl, Haagen-Smit, and Erxleben, 1934). So it is likely that the high initial content is inhibitory to maximum growth and that as the auxin concentration falls, the growth rate increases to a higher level. The reason for the growth rate of the roots in yeast extract remaining close to the initial level is not clear. It may be that the relative concentrations of vitamin B<sub>1</sub> and nicotinic acid are not optimum or that there are substances in the extract which are slightly inhibitory to growth. A third possibility is based on the fact that the Avena seedling is capable of changing tryptophane to indole-acetic acid. It may be that the pea root also forms auxin in this manner, from the tryptophane in yeast extract, thus augmenting its natural content to the point that growth is partially inhibited.

The effects of nicotinic acid on the anatomy of the root are now under investigation and will be reported in a separate paper. It is clear, however, that nicotinic acid, like vitamin  $B_1$ , must be supplied to

the roots if cell divisions are to take place in the meristem.

#### SUMMARY

An essential growth factor for excised pea roots, necessary in addition to vitamin  $B_1$  and present in yeast extract, was not found to be among the amino acids nor the microelements of plant nutrition.

Nicotinic acid can act as this growth factor. Salts, sugar, vitamin B<sub>1</sub>, and nicotinic acid can support the growth of pea roots indefinitely.

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### PACIFIC SECTION

Abstracts of the papers presented before the Pacific Section of the Botanical Society of America, Stanford University, California,

June 26 to 30, 1939

How does the little leaf disease affect the MERISTEM OF TREES? H. S. Reed, University of California, Berkeley, Calif.—Dwarfed shoots and leaves are characteristic of apricot and peach trees affected with the little leaf disease. The cytological aberrations of the meristematic region of affected shoots lead to hyperchromatization, vacuolization, and polarization of the cell contents. Tannic compounds are most abundant in early spring and late autumn. They are conspicuous in partially differentiated cells of the sub-apical region. All mitosis is inhibited in these cells. Phloroglucinol is most abundant in the phloem and medullary rays of affected apricot shoots about 5 mm. long in March when tannic compounds have disappeared. In June shoots of healthy apricot trees contain large amounts of phloroglucinol in the cortex, then in smaller amounts in medullary rays, pith and phloem. In general there is parallelism between the amounts of starch and phloroglucinol in the apricot shoots.

STUDIES OF THE DEVELOPMENT OF THE CARROT ROOT. Katherine Esau, University of California, Davis, Calif.—Vascular differentiation begins with the delimitation of a diarch plate through enlargement and vacuolation of the future vessels. Two protophloem sieve tubes are the first vascular elements to mature, and appear opposite each other next the pericycle. Then follows the differentiation of two protoxylem elements on the opposite marginal points of the future xylem plate. The usual centripetal differentiation of further xylem and phloem elements follows. A few centrifugal xylem elements complete the primary development. Oil ducts in the pericycle form two arcs each with the central duct located opposite a protoxylem pole. The hypocotyl resembles the root. In the cotyledons the endarch xylem appears first and the cotyledonary traces appear close below the insertion of the cotyledons. The lateral roots arise near the protophloem poles in the part of the pericycle that lacks oil ducts. In the lateral root development the endodermis forms a temporary covering over the young organ. The fleshy hypogeous storage organ of Daucus carota results from secondary growth in the root and hypocotyl. Cambium arises in the usual manner and forms highly parenchymatous secondary vascular tissues. The cortex is ruptured and shed. A parenchymatous periderm arises by active divisions in all pericyclic cells. Cork cambium and cork appear in the peripheral layers of the periderm outside of the oil ducts. The mature storage organ consists mainly of secondary vascular tissues, the periderm forming a narrow peripheral zone.

THE NATURE OF THE END WALLS OF VESSEL MOTHER CELLS. Katherine Esau and W. B. Hewitt, University of California, Davis, Calif.—The differentiating vessels of herbaceous angiosperms retain the partitions separating the individual elements until the latter reach their final volume. At this time each partition consists of two peripheral cellulose layers the two primary walls—and an isotropic intercellular substance between them. The end walls break

down when the cytoplasm disintegrates.

DEVELOPMENT OF THE FLOWER OF ANTIRRHINUM MAJUS L. Sam E. Keeton, University of California, Davis, Calif. (Read by Katherine Esau.)—The organs of the individual flowers appear in acropetal manner. In the calvx whorl the two abaxial sepals appear simultaneously, followed by the development of the three adaxial ones. The middle sepal of the upper trio is the last to form. The corolla lobes all become evident at the same time and alternate with the sepals. The tubular portion of the corolla results from the upward growth of a ring of tissue beneath the five corolla lobes. The epipetalous stamens alternate with the corolla lobes and appear shortly after the initiation of the corolla. The lower two stamens form first, followed by the two lateral ones, and lastly by the fifth upper stamen, which is normally suppressed at a very early stage. The bicarpellate ovary, imposed upon a fleshy disk, is the last floral organ to appear. The numerous anatropous ovules are borne on a thick axial placenta.

PRELIMINARY REPORT ON THE COMPOSITION OF THE CELL WALLS IN SPHAGNUM. George B. Rigg and Vida Goheen, University of Washington, Seattle, Wash.—The only substances found in the cell walls of Sphagnum are pectic substances indicated by a positive test with ruthenium red, and cellulose, indicated by its swelling and dissolving in cuprammonia following the removal of pectic substances with dilute oxalic acid and ammonium oxalate. Quantitative tests indicated that the content of pectic substances was above 60 per cent. The staining reactions should be supplemented by chemical analysis.

GERMINATION AND SEEDLING DEVELOPMENT OF CERTAIN DESERT PERENNIALS. T. D. Mallery, Desert Laboratory of the Carnegie Institution of Washington, Tucson, Ariz.—Seeds of ninety-four desert perennials representing thirty-four families were planted in soil under greenhouse conditions. The cultures were observed for germinative response and seedling characteristics and development. Fifty-one species germinated. Most species gave a very erratic germinative response. New seedlings appeared from time to time over a period of weeks. The juvenile forms of several species look quite unlike the mature plants. This is strikingly true of Holacantha Emoryi and Canotia Holacantha which change from a leafy to a leafless form. Practically all of the seedlings grew well in the greenhouse where conditions were considerably more mesic than out-of-doors.

THE FOUR STRAND STRUCTURE OF MEIOTIC CHRO-MOSOMES IN ALOE AND LILIUM. G. A. McCallum. Stanford University, Calif. (Introduced by L. L. Burlingame.)—The diakinesis chromosomes of Aloe striata contain two chromatids and each chromatid consists of a pair of twisted chromonemal threads. The metaphase coiling obscures these chromonemata. At anaphase the chromatids are in diads and each contains a coiled chromonema which is visibly double at late anaphase and early telaphase. In Lilium parvum the chromosomes show four chromonemata at diakinesis.

NEW AND INTERESTING RHODOPHYCEAE FROM SOUTHERN CALIFORNIA. George J. Hollenberg, La Verne College, La Verne, Calif.—A species of Dermocorynus, very close to D. Montagnei, the type species, has been found along the coast of Southern California. The latter seems not to have been collected since first described in 1867. Two new members of the Champiaceae are close to Gastroclonium, from which they both differ in the regular production of polyspores.

Some watermolds found in southern califor-NIA. James V. Harvey, San Bernardino Valley Junior College, San Bernardino, Calif.—Since December, 1935, 345 samples of water or soil have been taken in California, Arizona, and Utah for the purpose of isolating species of the Saprolegniaceae. No Saprolegniaceous species was obtained from fifty samples from the Arizona-Utah region, but Allomyces arbuscula Butler was taken from Totem Pole Wash in Monument Valley. Pythium species, mostly P. deBaryanum, various Zygomycetes and Ascomycetes, as well as Fungi Imperfecti and Algae were commonly isolated over the entire area. From the 295 collections made in California the following watermolds have been isolated, the number of times each organism was isolated following its name: Saprolegnia ferax, form I, Minden, 49; S. ferax, form II, Minden, 2; a new variety of S. delica Coker, 5; S. diclina Humphrey, 1; two undescribed, non-sexual species of Saprolegnia, 3, and 20 times, respectively; Achlya flagellata Coker, 3; A. caroliniana Coker, 7; A. sp., resembling both A. flagellata and A. caroliniana, 2; two types of Achlya without sexual reproduction, 11; Dictyuchus sterile Coker, 17; Dictyuchus sp., 1; Aphanomyces laevis DeBary, 19; Brevilengia megasperma Harvey, 2; Geolegnia inflata Coker, 1. The morphological features of each species isolated were illustrated with several lantern slides of line drawings.

INDUCTION OF POLYPLOIDY AND STERILITY IN AM-PHIDIPLOIDS INDUCED BY HETERO-AUXIN TREATMENT. Walter H. Greenleaf, University of California, Berkeley, Calif. (Introduced by R. E. Clausen.)-By means of the decapitation-callus method, using 1 per cent by weight of hetero-auxin (indole-3-acetic acid) in lanolin, 1,973 callus shoots produced by F<sub>1</sub> hybrids of N. sylvestris-tomentosiformis and by N. sylvestris-tomentosa, were classified. Of these, 14.7 per cent were tetraploids, about 1 per cent octoploids, and about 0.1 per cent otherwise unbalanced chromosomally. Autotetraploids of N. tomentosiformis, N. tomentosa, N. sylvestris, and tetraploids of N. glauca shoots were similarly obtained. The allopolyploids N. sylvestris-tomentosiformis and N. sylvestris-tomentosa proved completely female sterile, though the pollen was good. Cytological study indicates that this sterility is due to early degeneration of the embryo sacs. The pollen tubes grow readilv. Megasporogenesis is regular, so the degeneration cannot be ascribed to incomplete chromosomal complements resulting from disturbed chromosomal relations. The degeneration phenomena, essentially alike for both amphidiploids, are similar to that of the F<sub>1</sub> hybrid, which, however, exhibits abnormal meiosis. It is possible that D. Kostoff's fertile amphidiploid sylvestris-tomentosiformis, obtained by crossing N. sylvestris-tomentosiformis  $\times$  N. sylvestris X N. tomentosiformis, is a somewhat altered amphidiploid, possibly occurring on account of pairing in the F<sub>1</sub> hybrid sylvestris-tomentosiformis.

FLOWER COLOR-INHERITANCE IN THE CARNATION. Gustav A. L. Mehlquist, University of California, Berkeley, Calif. (Introduced by E. B. Babcock.)— Six independent factors concerned with self-colors have been identified. Their actions may be summarized as follows: Y controls the production of yellow anthoxanthin. I, epistatic to Y, controls the production of ivory anthoxanthin. A is the basic anthocyanin factor. It is normally effective only in the presence of both Y and I. Partial anthocyanin is possible in the absence of I, the resultant color varying from orange to maroon depending on what specific anthocyanin factors are present. S controls the production of scarlet-red anthocyanin; its recessive allele s produces dilute red or salmon. R controls the production of crimson-red anthocyanin; its recessive allele r permits the production of scarlet-red anthocyanin only. M modifies the red anthocyanin colors to the corresponding colors of the magenta series. It has no effect in the acyanic series.

(The following four papers were presented at a joint session with the Ecological Society of America, Western Section, in a symposium on "RECENT CONTRIBUTIONS OF BOTANY AND ECOLOGY TO SOCIETY.")

RECENT ACHIEVEMENTS IN PLANT BREEDING. E. B. Babcock, University of California, Berkeley, Calif.—Progress in the application of the Mendelian principles in plant breeding was reviewed under the following heads. (a) Breeding new varieties to order. (b) Utilizing hybrid vigor. (c) Breeding for disease resistance. The development of cytogenetics and

some important applications were discussed as follows: (a) Interspecific hybridization and amphidiploidy in plant evolution and breeding. (b) Artificial doubling of the chromosomes and the utilization of interspecific hybrids. (c) Using monosomics and trisomics in the mapping of chromosomes and its bearing on the breeding of the future. Improvements in technique involve, (a) Applied statistics, field plot technique, etc. (b) Determining chromosome numbers. (c) Speeding up normally slow plants.

SOME RECENT CONTRIBUTIONS OF ANIMAL ECOLOGY TO HUMAN WELFARE. Tracy I. Storer, University of California, Davis, Calif.—Problems of human welfare as affected by animals are numerous in the fields of agriculture, fish and game, commercial fisheries, forestry, fur production, public health, and recreation. Man has long sought to regulate, use, or destroy animals to his own benefit, though often without an accurate understanding of the interrelationships between animals and their environments. Qualitative information available has proved inadequate and is gradually being supplemented or replaced by quantitative data. Ecological relations of animals are perhaps the most complex phenomena of science, rivalling those of sociology, because of the multitude of interacting and often uncontrollable variables. Present day research in animal ecology, much of it with economic objectives, is gradually providing important material that will eventually portray truthfully the interrelations that occur in nature. Examples discussed included population studies in species with both stable and fluctuating numbers; effects of irrigation and of drainage on wild life; manipulation of cover as affecting game and non-game species; some newer knowledge of predator-prey relationships as the basis for more rational management: and some results proceeding from more detailed knowledge of rodent vectors of disease as affecting the health of man.

MYCOLOGY IN THE SERVICE OF MANKIND. E. P. Meinecke, U. S. Forest Service, San Francisco, Calif.—From oldest antiquity man has used fungi in some form for a variety of purposes. With the crystallization of knowledge about fungi in modern mycology and the refinement in scientific and industrial technique came an ever increasing utilization. It was in particular the development of the pure culture that made progress possible. The cultivation of fungi for food, highly developed on an empirical basis by the Chinese and Japanese, has grown into a large industry that works with pure strains and laboratory methods. Pure cultures are employed in the manufacture of such cheeses as Roquefort and Gorgonzola. Yeast in pure culture has become a popular treatment in certain nutritional diseases of man. The most important services rendered by mycology lie in the field of indirect uses in industries. With very few exceptions this is the only field of applied botany in which a plant is deliberately set to work to produce by-products rather than to produce plant tissue, flowers, or fruit. The control of harmful fungi, based mainly on a clearer understanding of fungous ecology and physiology has helped to make modern agriculture. In forestry, in horticulture, in the grain-field, the orchard and the greenhouse, success hinges upon a well-understood, carefully planned and executed control of fungous diseases. Research on the manifold mycoses has cleared up many of the formerly mysterious diseases of man and animals.

SOME RECENT CONTRIBUTIONS OF PLANT ECOLOGY TO HUMAN WELFARE. H. L. Shantz, U.S. Forest Service, Washington, D. C .- America has recently been aroused by the realization that cultivated fields, forests, grasslands, desert shrub areas, swamps, lakes, and rivers have been despoiled by careless overuse and abuse. These lands and waters must be brought back to a more normal and naturally productive state. The ecological approach is basic to all management of these areas. This is especially true of raw land use and of the management of forests, grasslands, deserts, swamps, lakes and streams. Farmlands and pastures likewise are receiving special attention from ecologists. Ecologists, by using the synthetic method on data obtained earlier by analytical investigations, are aiding in solving problems of soil erosion; grazing and range management; rebuilding depleted lands and waters to productive levels; control of weeds and succession of annual plants on areas under use; and general land, stream, and lake management. These are only a few of the practical problems confronting the plant ecologists.

THE EFFECT OF ANAEROBIC CONDITIONS ON MITOSIS IN SEEDLINGS OF HORDEUM. G. L. Stebbins, Jr., and Lotti Steinitz, University of California, Berkeley, Calif.—Actively growing seedlings of Hordeum vulgare were subjected to an atmosphere nearly free from oxygen by the use of nitrogen and pyrogallol. This caused immediate cessation of growth and of mitotic divisions. After three to four days mitosis was resumed under anaerobic conditions for a few days. The dividing tissue under these conditions shows the following differences from normal, control material. (1) The resting nuclei are smaller; (2) the staining is more intense, particularly of nucleoli and heterochromatic material, both in acetocarmine smears and in sections stained with gentian violet; (3) the chromosomes are much more contracted and tend to become clumped together at metaphase in both smear and sectioned preparations; (4) the chromosomes are often scattered through the cell at anaphase. Under prolonged anaerobic conditions the nuclear membrane breaks down, nuclear material becoming extruded into the cytoplasm. Some seedlings treated for four and five days recovered upon being returned to normal conditions, and after one to two days mitosis was normal in them.

STRUCTURE AND GROWTH OF THE SHOOT APEX OF CYCAS REVOLUTA AND ZAMIA FLORIDANA. Adriance S. Foster, University of California, Berkeley, Calif.—For the complete paper, see Amer. Jour. Bot. 26: 372–385.

THE USE OF AN ALTERNATING "TRANSLONGITOME"
IN MAKING AND INTERPRETING SERIAL SECTIONS.

D. M. Crooks, University of Arizona, Tucson, Ariz. -An alternating two-plane cutting attachment known as a "Translongitome" has been developed for use in an ordinary rotary or sliding microtome. which will cut alternate cross and longitudinal sections from the same block of tissue so that the alternate sections come from the microtome knife in one continuous ribbon. This ribbon, which has both cross and longitudinal cuts from the same block, may be mounted and examined on the same slide. If one follows a series of these sections, it is possible to observe a particular bundle or structure in cross section and also see the same bundle in longitudinal section. As any particular bundle in cross section comes to the cutting face of the longitudinal section, it is possible to determine the thickness of the bundle by counting the number of longitudinal sections which are required to cut through the entire thickness of the bundle and multiplying by the thickness of the section. It is necessary to set the microtome at onehalf the desired thickness for sections are cut from either face only on alternating strokes of the microtome. The translongitome is mechanically simple, and a manufacturing concern will soon make it available in a non-ferrous metal to those interested in its

A PLANT GROWTH INHIBITOR AND PLANT GROWTH INHIBITION. Wm. S. Stewart, California Institute of Technology, Pasadena, Calif.—When eight-day-old radish cotyledons are ether extracted according to the method given by Van Overbeek for auxin extraction, a non-toxic substance is obtained which is capable of causing plant growth inhibition. In the Avena coleoptile the inhibition is manifested as a positive growth curvature within 90 minutes from the time of application of the extract. The relation between the concentration of the inhibitor and the degrees of positive curvature is linear between 3 and 13 degrees. This allows the use of a modified Avena test as a quantitative biological assay for inhibitor substance. Using this method it was found: (1) That inhibitor was transported non-polarly in the radish and Avena plant; (2) that during the growth of the foliage leaves inhibitor disappeared and was replaced by auxin; (3) that inhibitor can be readily hydrolysed to form auxin, probably the auxin being indole-acetic-acid. Other experiments show that the positive Avena curvatures may be explained on a basis of the decreased growth rate of the inhibited plants.

A STUDY OF THE PERISPORIACEAE, CAPNODIACEAE, AND SOME OTHER "SOOTY MOLDS" FROM CALIFORNIA. Vera M. Miller, University of California, Berkeley, Calif.—"Sooty molds" form a superficial dark film of mycelium over the outer surfaces of plants. Many of these fungi are epiphytes and obtain their nutrition from the moisture of the air and from "honey dew" secreted by insects. Economically "sooty molds" are of slight importance. A number of "sooty molds" have been collected in the San Francisco Bay region where climatic conditions are particularly favorable to their growth. The perisporiaceous and

capnodiaceous "sooty molds" occurring in California were enumerated, with particular attention being given to previously undescribed species. A new genus, seven new species, and a new combination were proposed. Twenty-nine host plants were listed, some of the plants being attacked by as many as five species of "sooty molds." Twenty-eight "sooty mold" fungi are recorded. Several of them occur on more than one host, one of them appearing on six different plants—on three broad-leaved trees, on two ferns, and on redwood.

Effect of shade on flowering of sugar beets. Eubanks Carsner, U.S. Department of Agriculture, Riverside, Calif.—Fall planted beets at Riverside, California, bolted more extensively if artificially shaded. Vertical shades which protected the plants and the soil around them from direct sunlight, and horizontal shades which allowed the leaves to be exposed but shaded the surrounding soil, both resulted in increasing the percentage of bolters over that in unshaded checks. Shading the soil keeps it cooler. The temperature of the beet roots is nearly the same as that of the surrounding soil. Growth of beets at low temperatures is conducive to induction of flowering. Early planting gives nearly the same effect on bolting as does artificial shade, because a large leaf area developed in the fall effectively shades the soil during the winter. In addition to the indirect relation of leaf area to thermal induction of flowering there may be a relation between leaf area and induction by light.

Aposporic development in crepis. G. L. Stebbins, Jr., and J. A. Jenkins, University of California, Berkeley, Calif.—Reproduction in the polyploid forms of the North American species of Crepis is by means of somatic apospory followed by diploid parthenogenesis. Meiosis in the pollen mother cells of three apomicts of C. occidentalis and C. intermedia is nearly normal except for the occurrence of multivalents, and of bridge-fragment configurations at first anaphase. In an apomict of C. acuminata the pollen mother cells degenerate in mid-prophase of meiosis so no pollen is formed. The various apomicts differ in the stage of development at which apospory begins and in the frequency with which it occurs, but in all but one of those studied normal embryo sacs were formed in some of the ovules. Apomixis is therefore chiefly facultative and resembles closely the type already known in Hieracium subg. Pilosella.

INHERITANCE OF RED SEED COAT COLOR IN COM-MON BEANS. Francis L. Smith, University of California, Berkeley, Calif. (Introduced by E. B. Babcock.)—In a search for red seed coat color genes and modifiers which could later be used in a program of breeding improvement, a number of independent genes were found. Results of numerous crosses and the effects of the genes involved were reported. Many of the results were difficult to interpret.

ANATOMY OF VASCULAR TISSUES. Katherine Esau, University of California, Davis, Calif.

Translocation of viruses in plants. C. W. Bennett, U. S. Department of Agriculture, Riverside, Calif.

MOVEMENT AND DISTRIBUTION OF RADIOACTIVE ELEMENTS IN PLANTS. D. R. Hoagland and J. P. Bennett, University of California, Berkeley, Calif.

THE MECHANISM OF MOVEMENT OF ORGANIC MATERIALS IN PLANTS. (a) PROTOPLASMIC STREAMING. O. F. Curtis, Cornell University, Ithaca, New York. (Read by A. S. Crafts.) (b) ACTIVATED DIFFUSION. T. G. Mason, Cotton Research Station, Trinidad, British West Indies. (c) PRESSURE FLOW. A. S. Crafts, University of California, Davis, Calif.

The six papers listed above were presented before a joint session of the Botanical Society and the Western Section of the American Society of Plant Physiologists in a symposium on "The Translocation of Solutes in Plants." The abstracts are in the hands of the secretary of the latter society.

(The following five papers were presented in a symposium on "Native Plants of Western North America Offering Exceptional Material for Botanical Research.")

THE LOWER PLANTS, ESPECIALLY ALGAE. Gilbert M. Smith. Stanford University, Calif.—Intensive investigation of small ponds, reservoirs, and streams offer excellent opportunities to the algologist interested in fresh water forms, though that flora is less rich in the western United States than in some of the regions where numerous lakes provide suitable habitats for the smaller fresh water algae. The outstanding opportunity seems to lie in the investigation of the vegetative features of the organism responsible for "red snow," but few have seen the rarer phenomenon, "green snow." The latter should be studied. Careful collecting and study of the marine algae should yield valuable information concerning the life histories and range of the smaller, less known species. The work of G. J. Hollenberg demonstrates the fruitfulness of this type of painstaking work. Collectors studying the Heterocontae and Chrysophyceae should use hard glass containers in order to avoid disintegration of delicate algae, many of which are very sensitive to minute traces of substances contained in soft glass.

OPPORTUNITIES FOR RESEARCH IN MYCOLOGY IN CALIFORNIA. Lee Bonar, University of California, Berkeley, Calif.—Owing to the wide range of climatic conditions and to the relationship of the fungi to the higher plants of the region, the mycoflora of the state is rich in variety and number of species and in the wide range of different types. A comparison of the status of our present knowledge of the seed plants with that of our mycoflora emphasizes the need for systematic collection and study of the fungi. The disposition of collections of early workers was such that few are available locally. It is important that we build up collections of western fungi in herbaria in our area. Monographic studies of certain groups show that many species are peculiar to our region. Many other groups are well represented in the state and offer exceptional material for investigation. The study of incompletely collected areas entails long continued, intensive, and repeated collections. The fungi of the arid regions should be studied and compared with those of similar habitats in other parts of the world. Fungi parasitizing introduced seed plants should be studied. Good opportunities exist in the field of cultural, inoculation, and morphogenesis studies.

PLANTS OF THE SOUTHWEST OFFERING OPPORTU-NITY FOR BOTANICAL RESEARCH. D. M. Crooks, University of Arizona, Tucson, Ariz.—The range grasses offer many opportunities for anatomical work in interpreting their life histories relative to environment and grazing practices. Marked variations in different species are indicated by preliminary studies and the relation of establishment of adventitious root systems to climatic conditions needs study. Detailed studies of life histories of certain range weeds will be useful in determining methods of control. Studies of the floral development in Burroweed, in which flower primordia initiated in August mature seed in sixty days, serve as a guide and beginning point in this type of work. The problem of the lack of nodule development on the roots of desert leguminous plants should be investigated from a morphological viewpoint. Morphological studies should be undertaken in connection with the form of growth, gross development, and ontogeny of parts of native plants exhibiting habits very different from those of plants growing in more mesic conditions. Six main types of desert plants invite this sort of investigation. They are: (1) those with succulent stems; (2) thorny shrubs with reduced leaves and specialized stems; (3) chaparral type of shrubs; (4) semiherbaceous perennials with peculiar types of secondary root development; (5) the fleshy storage root type; (6) thick stemmed monocotyledons.

CYTOGENETICS, INTERSPECIFIC RELATIONSHIPS, AND DISTRIBUTION IN THE WESTERN FLORA. G. L. Stebbins, Jr., University of California, Berkeley, Calif.—The application of cytogenetic techniques to the species problem has made possible the recognition of several cytogenetic patterns which are correlated with patterns of morphological variation. All of these occur in genera in the western flora. The types are: (1) Rassenkreis. The genus contains relatively few species, each with the same number of chromosomes, but each species consists of several interfertile subspecies or geographic varieties. (2) The homoploid complex. Genera of large numbers of species separated by slight morphological differences, but more or less intersterile in spite of uniform chromosome number. (3) The aneuploid series. Closely related species have different basic chromosome numbers; otherwise like (2). (4) The polyploid complex. Species of the group have different chromosome numbers that are multiples of the same basic number. Diploid species are distinct from each other, but are connected by a series of auto- and allopolyploid derivatives. (5) The agamic complex. Similar to (4) but the polyploids partially or wholly apomictic. (6) The segmental interchange complex. All species with the same chromosome number but with different segmental arrangements, with races that form rings or chains at meiosis common. Hybrids among these races may retain their constancy by means of a system of balanced lethals. A study of a number of such groups in the western flora should contribute materially toward an understanding of its history.

EXPERIMENTAL STUDIES ON RELATIONSHIPS OF WESTERN PLANTS. William M. Hiesey, Carnegie Institution of Washington, Stanford University, Calif. —A review of the transplant work carried on by the Carnegie Institution staff at the three stations located at Stanford University, at Mather, and at Timberline in California, indicates the possibilities in this field. The West, with its varied topography and climates, offers exceptional opportunity for much-needed investigations on the relationship between plants and their environment. Analyses of the composition of ecotypes would now be especially valuable to complete our understanding of species composition. A field offering much promise is the comparative anatomical and physiological study of ecotypes of one species, or of closely related species.

THE PROCERI PENSTEMONS-A PROBLEM IN CYTO-TAXONOMY AND DISTRIBUTION. David D. Keck, Carnegie Institution of Washington, Stanford University, Calif.—Penstemon, of the Scrophulariaceae, is a genus composed of many sections and subsections. The subsection Proceri contains some of the most complex taxonomic problems in the genus. The great number of forms which have been ecologically differentiated within it is now better understood as a result of a cytological survey of many of the species and races and from transplant experiments. Apparently the center of origin of the Proceri Penstemon species is in the Columbia River basin, from which a radiation of forms has occurred in all directions. Most of the species are diploid (n = 8), but there are obviously related tetraploid and hexaploid species, which are apparently derivatives of the diploids and occupy different habitats from them.

The limits of the plant kingdom. Herbert F. Copeland, Sacramento Junior College, Sacramento, Calif.—The proposal made recently (The Kingdoms of Organisms, Quart. Rev. of Biol. 13: 383–420, 1938), to recognize two kingdoms of organisms, designated respectively as Monera and Protista, in addition to the Plantae and Animalia, was discussed. A summary of comments and criticism communicated to the author indicated an almost equal division between support and opposition to the proposal.

The taxonomy and geographic distribution of the genus erysimum in north america. George B. Rossbach, Stanford University, Calif.—The genus Erysimum of the family Cruciferae is a variable group occupying sterile, sandy, or gravelly habitats through much of the northern hemisphere. Fifteen species and seven varieties, some of which include taxonomic complexes, are recognized. Geographical distributions of entities may be circumboreal, or in North America, may comprise such large areas as the higher mountains of the western United States,

or the Great Plains. Others may be restricted to local areas, such as certain coastal parts of Quebec and Newfoundland; the higher Olympic Mountains of Washington; the San Francisco Peninsula in California; the marine sand deposits on the southern edge of the Santa Cruz Mountains; small areas of coastal dunes; or the Channel Islands off the coast of southern California.

Thomas howell, the pioneer botanist of oregon. Albert R. Sweetser, University of Oregon, Eu-

gene, Ore. (Read by title.)

GEOGRAPHICAL DISTRIBUTION AND ECOLOGY OF SOME NORTH AMERICAN SPECIES OF DESCURAINIA, AND THEIR BEARING ON THEIR TAXONOMY. LeRoy E. Detling, University of Oregon, Eugene, Ore.—Owing to the wide range and adaptation to greatly varying habitats several species of Descurainia in North America offer exceptional opportunities for studying the selective effect of habitat on the species. Each climatic niche in western North America is occupied by one particular form of the species. Each form so limited by its environment is considered a subspecies. In D. pinnata subspecies have developed in: (a) South Atlantic and Gulf coasts; (b) plateau regions of the southwestern states; (c) deserts of southwestern United States and adjacent Mexico; (d) southern California coastal region; (e) the northern borders of the Great Basin; (f) the plains of the north central states and Canada; (g) the central Rocky Mountains; (h) Columbia Plateau; (i) desert region of Wyoming and Utah. The subspecies of D. Richardsonii occur in moister, cooler habitats located in: (a) Canadian plains; (b) central Rocky Mountains; (c) mountains to the east, north, and west of the Great Basin; (d) southern Rocky Mountains. D. obtusa consists of three subspecies occurring in: (a) juniper belt of the Colorado Plateau; (b) deserts of the southwestern United States; (c) pine forests included within the range of the two above.

THE TAXONOMIC SIGNIFICANCE OF THE NECTARY SCALE IN RANUNCULUS. Lyman Benson, University of Arizona, Tucson, Ariz.—Five types of nectary scales are found in the North American species of Ranunculus. Some types are characteristic of whole sections. (1) Simple scales, free laterally and overarching the gland: subg. Euranunculus sect. Chrysanthe (except R. recurvatus); most species of sect. Echinella; subg. Cyrtorhyncha, sect. Halodes; subg. Ceratocephalus. (2) Pocket type, attached laterally as well as basally: subg. Euranunculus sect. Flammula and Epirotes; subg. Cyrtorhyncha sect. Arcteranthis and Pseudaphanostemma; subg. Batrachium; subg. Pallasiantha; subg. Coptidium; subg. Ficaria. (3) Low transverse ridge neither forming a pocket nor overarching gland: subg. Cyrtorhyncha sect. Eucyrtorhyncha; subg. Oxygraphis. (4) Transverse ridge with lateral ends produced into flaps extending distally and attached to the surface of the petal, the flaps sometimes meeting and then surrounding the gland: subg. Euranunculus sect. Hecatonia (some species); subg. Crymodes. (5) The scale bearing the nectary in a pocket on its own ventral surface: R. flabellaris (delphinifolius), and sometimes R. Purshii. Special peculiarities, such as the ciliate scales of R. cardiophyllus and R. arizonicus, are helpful in determining the limits of species.

STRUCTURAL HYBRIDITY IN PAEONIA CALIFORNICA AND P. BROWNII. G. L. Stebbins, Jr., University of California, Berkeley, Calif. (For a paper dealing with these species see Madrono 4: 252-260. 1938.)

WESTERN AMERICAN ACHILLEA AS DEFINED BY EX-PERIMENTAL TAXONOMY. Jens Clausen, Carnegie Institution of Washington, Stanford University, Calif. -The Western American Achilleas of the millefolium complex are native, not introduced. This complex, which encircles the northern hemisphere, has probably attained its greatest evolutionary differentiation in the Western United States. It has here evolved ecotypes adapted to climates ranging from the maritime to the arctic-alpine and the dry Great Basin conditions. Contrary to the general assumption, our West American Achilleas are specifically distinct from the Eurasian Achillea millefolium. We have at least two indigenous species. One is the hexaploid Achillea borealis Bong. (n = 27), which occurs along the Pacific Coast of North America from Alaska to Lower California, occupying also the Coast Ranges of California. The other is the tetraploid A. lanulosa Nutt. (n = 18), which occurs from low to alpine altitudes in the Sierra Nevada and the Cascades and east through the Great Basin to the Rockies. Each of the two contains several morphologically very diversified ecotypes, which react very differently when transplanted to diverse altitudes. The Northern European A. millefolium L. is hexaploid like borealis but does not hybridize with it.

Hybrids of the california flora. Carl B. Wolf, Rancho Santa Ana Botanic Garden, Anaheim, Calif.

—A large number of supposed hybrids have been reported as forming part of the California flora but few have been tested by cytological or breeding experiments to determine their exact status. It is suggested that some of the puzzling complexes in our flora might be cleared up by critical field observations and garden experimentation. Collectors should try to take representative samples in a variable population instead of selecting, somewhat unintentionally, the plants that make the most convenient herbarium specimens.

PROBLEMS OF DISTRIBUTION AND VARIATION IN THE GENUS ZYGADENUS. Oliver S. Walsh, University of California, Berkeley, Calif.—Great variations occur in the gross morphology of Zygadenus under different environmental conditions. Wet habitats promote woody growth, large leaves, large flowers; dry situations tend to encourage smaller plants, leaves, and flowers, succulent bulbs, and a more solitary growth habit.

THE GENUS MONOLOPIA: A PROBLEM IN DISTRIBUTION AND DIVERGENCE. Ethel Crum, University of California, Berkeley, Calif.—Monolopia, belonging to the tribe Helenieae, is endemic in California and,

excluding *Pseudobahia*, consists of four species having their center of distribution in the south Coast Ranges. *Monolopia* seems to be the resultant of three lines of reduction common in the *Helenieae*: (1) loss of pappus; (2) loss of receptacular bracts; (3) reduction in number of involucral bracts. A secondary

line of divergence manifests itself in the development of a slightly bilabiate ray corolla. Important tendencies toward divergence within the genus itself are: union of the involucral bracts; flattening of the achenes. One of the four species included is a previously undescribed entity.

# ALCOHOL EXTRACTION OF GROWTH HORMONE FROM PLANT TISSUE 1

George S. Avery, Jr.

Two general methods are in use for assaying the growth hormone in plant tissues—the diffusion method (Went, 1928) and the extraction method (Thimann, 1934; Laibach and Meyer, 1935; Boysen Jensen, 1937; du Buy, 1938; Overbeek, 1938a). The former makes it possible to measure the amount of hormone that will diffuse from tissue into agar, and this has been interpreted as indicating the relative concentration of hormone in the tissue. The extraction method, on the other hand, makes possible an assay of the total quantity of hormone in the tissue or organ being tested. Both methods have their place, according to the problem being investigated; but the latter, as a truly quantitative method, marks an important advance in plant hormone studies.

Aside from the extraction method used by Laibach and Meyer (1935), all others utilize chloroform or ether as the solvent. In contrast to the chloroform and ether extraction methods, Laibach and Meyer boiled plant tissue in acidulated 96 per cent alcohol in order to extract the plant growth hormone. For purposes of testing, the concentrated extract was mixed with lanolin and applied unilaterally to Avena coleoptiles (not decapitated).

The extra time involved in extraction of growth hormones from impervious materials, such as seeds, and the possible loss of heat-labile growth promoting hormones by the Laibach and Meyer method, or the ether or chloroform destruction of them by the other procedures, led to the development of a method for their rapid extraction from ground plant material with cold absolute alcohol. Possible oxidation reactions destructive to growth hormones are prevented by the use of alcohol and the immediate grinding of the tissues. The method is based primarily upon extraction methods which are known to remove many pigments rapidly and completely from plant material (Strain, 1938). The procedure outlined here has been used for the past eight months; it gives reproducible and reliable results. Thus far it has been used for the extraction of growth hormone from maize endosperms and dormant maize seeds. The following is an outline of the method:

A. MATERIAL AND PREPARATION.—Ten maize seeds (about 3 grams) plus an approximately equal weight of sand2 plus five to ten times seed weight of absolute ethanol are ground in a glass mortar until no particles are visible. N/10 HCl may be added to the amount of one-fifth the volume of ethanol, though this apparently has no per-

<sup>1</sup> Received for publication April 17, 1939.

It is a pleasure to express my appreciation to Dr. H. H. Strain of the Carnegie Institution, Stanford University, California, for his suggestion of the method and his inter-

est in its development.

<sup>2</sup> Sand. Treat with 30 per cent NaOH two hours. Wash thoroughly with water. Wash with concentrated HCl overnight. Wash in running tap water 10 hours. Allow to stand in water and test with litmus. Wash with distilled water. Drain and dry before using as indicated above.

ceptible effect on extractions from dormant seeds. A single maize seed may be extracted by the same method, using correspondingly smaller portions of sand, ethanol, etc.

B. FILTRATION.—The ground mass is placed on a filter of Hyflo Super Cel (Johns-Manville) prepared as indicated in figure 1, using a funnel just large enough so that all the



Fig. 1. Diagram showing round bottom flask and funnel prepared for filtering tissue ground with ethanol.

ground material can be added at once. Suction is then applied. The mortar and pestle are washed with two 10 cc. portions of ethanol, pouring each through the filter, using suction. (Note: When tissue ground with ethanol is poured into filter, distribute evenly on the sand and allow to stand for a short time before starting suction.)

C. EVAPORATION OF FILTRATE.—After filtering, remove the stopper with funnel (apparatus indicated in fig. 1), and insert a one-hole stopper with glass tubing, for suction attachment. Evaporate filtrate at reduced pressure with water bath at 50-55°C. Temperatures up to 80-90°C. have not proven destructive to growth hormones extracted from maize seed, hence in this instance it is not important to keep temperatures low. In extracts of many materials, however, it would seem best to keep the temperature low in case there should be volatile or decomposable growth hormones present.

D. GROWTH HORMONE-AGAR MIXTURE.—To the dry residue obtained by evaporation of alcohol, add 20 cc. of water,3 and shake well. (For small amounts of tissue it may be necessary to add as little as 2-4 cc. of water; if so, add 2 cc. of recently distilled ether, shake well, and then warm in 50-55°C. water bath to evaporate ether. The addition of ether reduces the yield of growth hormone, hence it is better wherever possible to use larger amounts of tissue and take up the dry residue in water only.) Remove 1 cc. of water and dilute this with 1 cc. hot 3 per cent agar. The agar should be washed and allowed to soak in several changes of distilled water for four or five days before use, then dried, and made up at required percentages (1.5 and 3 per cent).

E. Dilution.—Use one part of the agar-water mixture (containing growth hormone) from D with one or more parts of 1.5 per cent agar, depending upon the dilution desired for testing. Several different dilutions should be prepared and tested to make certain that the test is within the "proportionality range" of the Avena test plants (see table 1). If the dilutions do not give curvatures in this range, the Avena tests should be repeated until the proper dilutions are determined for making possible a quantita-

tive assay.

F. CASTING OF AGAR PLATES .- Pipette the hot agar, containing given dilutions of growth hormone extract, into molds  $8 \times 10.7 \times 1.5$  mm. When the agar has set, cut into

<sup>3</sup> See table 1; 20 cc. is the proper amount in this instance. Actually, the dilution must be such that the hormone tests will be in the "proportionality range."

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Table 1. Hormone content of dormant endosperms, variety Canada Flint. Each figure representing Avena curvature at the different dilutions in series A, B, etc., is the average of twelve test plants. Each series represents the extraction of ten endosperms. Avena test laboratory maintained at 25°C. and 88-90 per cent humidity.

	Average weight of endosperm in each	Avena curvature in degrees by the "deseeded" method (Skoog, 1937) <sup>a</sup> Series					
Method number	series	cent agar to the equivalent of:	A	В	C	D	Average
I. Alcohol not acidulated. Dry residue taker up in 2 cc. of water and 2 cc. of ether before dilution	,	$\begin{cases} 40 \text{ cc.} \\ 80 \text{ cc.} \\ 160 \text{ cc.} \end{cases}$	25.0 11.2 7.5	29.4 14.1 6.0	··· 9.0	 6.2	27.2 12.6 7.2
II. Alcohol acidulated. No ether used. Dry residue taken up directly in 80 cc. of water, and 1 cc. made up with 1 cc. of 3 per cent agar	f f	(160 cc. )320 cc.	15.8 7.5	16.4 7.7	19.0 11.3	18.9 9.9	17.5 9.1
III. Alcohol not acidulated, otherwise as in II	2.5 g.	160 ec.	14.2	16.2	14.3	16.0	15.2

<sup>&</sup>quot;The endosperms are removed from test plants 16 hours before use. The coleoptiles are decapitated, and immediate unilateral application of agar blocks follows. Shadow photographs of curvatures are taken after five hours.

12 equal blocks for application to decapitated Avena coleoptiles.

Each experiment is run in two or more series, as in table 1, to insure an adequate check on the accuracy of the determinations. Typical results, obtained from the extraction of endosperms (embryos removed) of dormant grains of maize, variety Canada Flint (obtained through the courtesy of Dr. Ralph Singleton of the Connecticut Agricultural Experiment Station), are recorded in table 1. The Avena test plants were A. sativa, var. Victory (Svalöv).

Computation of results.—The total hormone content of the endosperms may be expressed in terms of total degrees curvature of *Avena* test plants, computed as follows:

The dimensions of each small rectangular plate of agar cast in the above-mentioned mold are  $8 \times 10.7 \times 1.5$  mm. (Dolk and Thimann, 1932), hence the volume is 128.4 cubic millimeters. Each piece  $8 \times 10.7 \times 1.5$  mm. is cut into 12 small blocks of equal size, hence whatever growth hormone is contained in the agar plate is capable of producing a given curvature in each of twelve test plants. In the case of method III, table 1, an average curvature of 15.2° was obtained. Tests on further dilutions showed the response

Table 2. Hormone content of dormant endosperms, variety Canada Flint. Each figure representing Avena curvature at the different dilutions in series A, B, etc., is the average of twelve test plants. Each series represents the extraction of one endosperm. Avena test laboratory maintained at 25°C, and 88-90 per cent humidity.

Method	Extract from one endosperm di- luted in 1.5 per cent agar to the	"deseeded" method (Skoog, 1937)  Series							
number	equivalent of:	A	В	C.	D	E	F	Average	
I. Alcohol not acidulated. Dry residue taken up in 2 cc. of									
water and 2 cc. of ether, be-	190 00	8.1	10.5	8.3	5.8	6.6	8.6	8.0	
fore dilution	40 cc.	4.4	5.4	1.5	3.3	3.3	2.8	3.4	
Weight per endosperm (grams)		.25	.26	.25	.23	.21	.25		
II. Alcohol acidulated. No ether used. Dry residue taken up directly in 10 cc. of water,									
and 1 cc. made up with 1 cc.		17.4	19.9	17.4	16.5	19.0	19.9	18.3	
of 3 per cent agar	\\ 40 cc.	10.9	7.7	10.9	9.4	10.7	7.7	9.5	
(grams)		.25	.25	.25	.25	.25	.25	S*	
III. Alcohol not acidulated, otherwise as in II Weight per endosperm	\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\\	10.3 5.3	16.9 9.2	10.4 7.2	3.7	10.1 3.6	10.1 7.8	11.0 6.1	
(grams)		.25	.25	.25	.25	.25	.23	B	

Table 3. Comparison of hormone content per gram of endosperm of dormant maize seeds, variety Canada Flint, when extracted in different amounts and by different methods (tables 1 and 2).

	Average curvature per endosperm		
	Calculated From from extrac- extrac-	Average weight per endosperm (grams)	Total degrees curvature per gram of endosperm
Method number	tion of ten tion of one endosperms (table 1) (table 2)	From From table 1 table 2 $(10)$ $(1)$	From From table 1 table 2 (10) (1)
I		0.26 0.24 0.26 0.25 0.25 0.25	41,181 62,329 99,400 136,768 91,176 82,208

<sup>&</sup>lt;sup>a</sup> Calculations based on 160 cc. dilution.

to be in the proportionality range. Hence, the computation is as follows:  $15.2\times12=182.4=$  total degrees curvature obtainable from 128.4 cubic millimeters of agar containing a given concentration of growth hormone.— The total volume of agar in this experiment is 160 cc., or 160,000 cubic millimeters.— $160,000\div128.4=1,246$ . That is, 160 cc. of agar would, if east in molds as above, make 1,246 agar plates  $8\times10.7\times1.5$  mm.—Hence 1,246  $\times$  182.4 = 227,270 total degrees curvature for ten endosperms, or 22,727 degrees curvature per endosperm. This means that the growth hormone present in each dormant endosperm of Canada Flint maize, if extracted by the method described, is capable of producing a total of approximately 22,727 degrees curvature in a suitable number of Avena test plants ("deseeded" method).

The above computation of results has the same basis as the plant unit of Dolk and Thimann (1932), where one plant unit is that amount of growth hormone which, when applied in an agar block of ca. 10 cmm. to a test plant, causes 1° curvature. The plant unit, however, is based on Went's "standard" method; curvatures in the "deseeded" method are approximately four times as great as in the "standard" method (Avery, Creighton, Hock, 1939).

When put on a weight basis, the results of the different methods may be readily compared (table 3). Method III gives the best agreement between the extractions of one and ten endosperms.

Discussion.—Skoog's "deseeded" method has been used here rather than the "standard" method of Went, because lower concentrations give Avena curvatures of greater magnitude, also because Koningsberger and Verkaaik (1938) report less fluctuation in day to day tests by this method.

It will be noted that the yield in method I is lower than in methods II and III. The loss in method I may be due partly to the use of ether and partly to adherence to the walls of the flask (because of the small volume of water and ether). In any case, it is clear that taking the dry residue up in a large amount of water is effective in increasing the yield. If necessary to use a small amount of water because of a small amount of tissue being extracted, as indicated in step "D," the flasks should be not more than 50 or 100 cc. capacity; this will reduce the loss to the walls of the flask.

If the residues are fatty or gummy, as they are in the case of maize seeds, the growth hormone may go into solution slowly, even when a large volume of water is used. Small glass beads or coarse quartz sand added to the alcohol about the time that fats start to separate, during the evaporation (step "C"), increases the surface so that the extraction of the final dry residue with water is more complete.

Acidulation of the alcohol used in extraction in step "A" increases somewhat the yield of growth hormone (from dormant seeds), particularly in the case of the extraction of one endosperm. This is very likely due more to the addition of water than to the addition of acid. Van Overbeek (1938a) has reported a higher yield (than that reported here) of hormone from maize by water extraction. Cholodny (1935) found no hormone when extracting maize seeds, etc., with alcohol, whereas water extraction gave positive results. Using the alcohol method described here, Avery, Creighton, and Hock (1938) have reported preliminary results comparable with certain of those of Cholodny, in that endosperm after a few hours hydration gave a greater yield of growth hormone than the dry endosperm.

An extensive comparative study of the numerous different extraction methods described in the literature will soon be completed.

#### SUMMARY

A method is described for the rapid extraction of all alcohol soluble growth hormone from ground plant tissues, using absolute ethanol. The method does not involve use of ether or chloroform, and extracts can be prepared quickly for testing by standard procedures, such as Skoog's "deseeded" method or the "standard" method of Went. To date exhaustive tests have been made only on maize endosperms and entire maize seeds.

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# A DEVELOPMENTAL ANALYSIS OF HETEROSIS IN LYCOPERSICON. II. THE ROLE OF THE APICAL MERISTEM IN HETEROSIS <sup>1</sup>

William Gordon Whaley

DIRECT MEASUREMENTS were made of the volumes of the apical meristems of the plants described in Part I of this paper (Whaley, 1939) in an attempt to test the validity of Ashby's hypothesis (1930, 1932, 1937) concerning the importance of the mass of "original meristematic capital" in the development of hybrid vigor. Cell and nuclear sizes in the meristems were studied in some detail to determine what correlation, if any, could be found in these embryonic masses between morphological changes during development and the behavior of the plant as a whole.

Methods.—Each time samples were taken for weight determinations, ten main stem tips of each type were collected. These were immediately killed in chrom-acetic-formaldehyde solution and imbedded in paraffin. Serial longitudinal sections, 6 microns in thickness, were cut through a stem tip of each type from each sample. Care was taken to preserve the sections in proper serial sequence. These were mounted and stained with a triple stain of Delafield's hematoxylin, safranin, and light green. Using a Leitz projection microscope, a series of drawings of the entire apical meristem at a magnification of 210 diameters was made for each stem tip. Usually about 25 sections were required to make a complete series through a meristem. Very little shrinkage was observable in any of the tissues.

Since comparative volume measurements of the apical meristem were one of the important objects of this study, it was essential to define the term for the purposes of the investigation and to be able to recognize as the meristem a region the extent of which could be clearly defined in the material. For the following discussion the term "meristem" may be defined as that area at the tip of the stem in which the cells are isodiametric and non-vacuolate, or in which the vacuoles are extremely small. The region <sup>1</sup> Received for publication April 24, 1939.

selected presumably represents that area in which, at division, at least one of the daughter cells always remains undifferentiated. It is thus the truly embryonic portion of the meristem and the region of most active division. There is some tissue outside this area in which cell division occurs, but in such cases differentiation has already begun. Observation of large numbers of sections leaves no question that the region selected does represent that of most active division and that its boundaries can be sharply delimited. The number of mitotic figures present, the more intense staining reaction, and the complete absence of any differentiated cells unite in distinguishing this region from the rest of the growing point. Meristems of the plants in cross I [Lycopersicon esculentum  $\times$  L. pimpinellifolium (Whaley, 1939)] are illustrated in figures 5-15.

Meristem volume was measured in the following manner: plates of uniform thickness of 1.3 mm. were made from a specially prepared modelling wax in a wax-plate machine which could produce them accurately to tenths of a millimeter in thickness. The thickness of the plates (1.3 mm.) was determined by multiplying the thickness of the original tissue sections by the magnification, taking the result to the nearest tenth of a millimeter. The projected drawings made from serial meristem sections were placed upon the wax plates, and by means of a steel needle cutting through both paper and wax, wax patterns exactly the size and shape of the drawings were secured. The wax sections made from each of the series of projection drawings for each individual stem tip were then weighed together. Knowing the specific gravity of the wax, it was possible to determine the volume of the plates making up each series. By dividing this volume by the magnification used, the actual volume of the meristem could be calculated. Although a certain amount of error may be introduced by this method, it seems to be the most satisfactory one possible. The results obtained are consistent and reliable enough for comparative purposes.

From the series of sections through each meristem, the median section, or what appeared nearest to a median one, was chosen for the measurement of cells and nuclei. From this section camera lucida drawings were made of cell and nuclear outlines, using a magnification of 1,850 diameters. Where there were

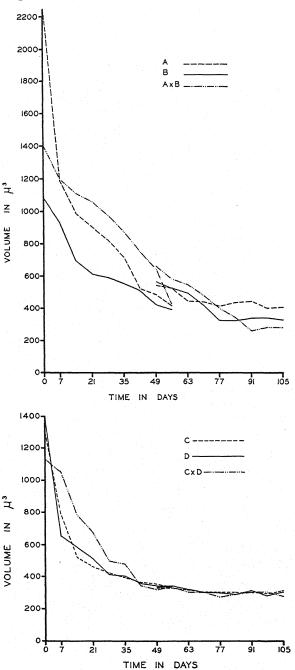


Fig. 1-2.—Fig. 1 (above). Cross I. Changes in cell volume during development. Time is given in days from planting.—Fig. 2 (below). Cross II. Same as figure 1.

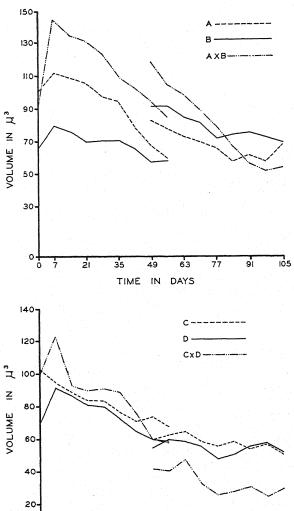


Fig. 3-4.—Fig. 3 (above). Cross I. Changes in nuclear volume during development. Time is given in days from planting.—Fig. 4 (below). Cross II. Same as figure 3.

TIME IN

49

63

DAYS

21

35

many cells, at least seventy were drawn, but where the cell number in a median section was small, as many as possible were drawn, in no case less than thirty-five. The cells in the apical meristem are irregular polyhedrons in shape but tend to be approximately isodiametric in outline. Volume was calculated by measuring the longest and the shortest diameter of each projected cell outline, determining the average, dividing by the magnification, and cubing the resulting value. This method, of course, gives the volume of the cell on the basis of a cube, and hence all volumes are probably somewhat too high as absolute measurements. The actual volume of polyhedral cells must lie between that of a cube and that of a sphere of equal diameter. The measurement of cells, and particularly of such small irregu-

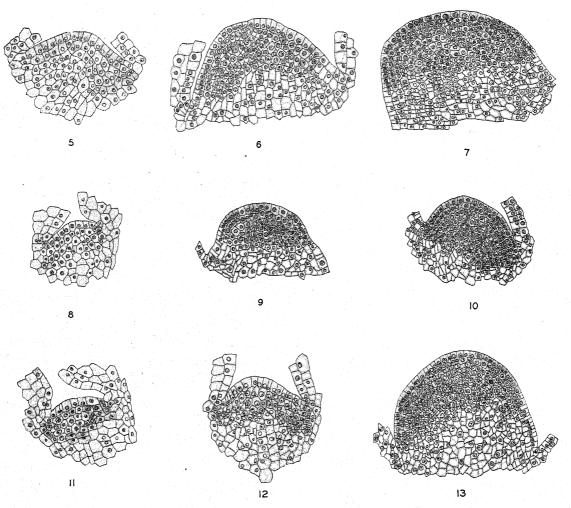


Fig. 5-13.—Fig. 5, 6, 7. Parent A. Camera lucida drawings of median longitudinal sections through the apical meristem in the seed, after 7 days' growth and after 77 days' growth, respectively, showing developmental changes in the size and shape of the meristem and in the size of its cells and nuclei.  $\times 133$ .—Fig. 8, 9, 10. Parent B. Same as preceding figure.—Fig. 11, 12, 13. Hybrid A  $\times$  B. Same as preceding figure.

lar cells as occur in the apical meristem, is extremely difficult. The results are, therefore, of doubtful validity as absolute measurements; but when large numbers are used, the results are satisfactory for comparative purposes. Nuclei were measured by the same technique used for the cells. As meristem nuclei are spherical, their volume was calculated by substituting diameter measurements in the formula for the volume of a sphere.

Cell number was obtained in each case by dividing the volume of the meristem as a whole by the volume of the cells. The results permit a comparison of relative cell numbers in any series at any time. They should not be taken, however, as absolute counts of cell number, partly because of the inadequacy of the cell size measurements, as shown above, and partly because to obtain accurate actual counts it would be necessary to take into account the difference between the dermatogen cells and the remainder of the meristem. The former tend to remain con-

stant in size during development. In no case were any of these included in the cells measured to determine cell volumes.

MERISTEM VOLUMES.—The meristem volume measurements are given in table 1. The entry at zero days is the volume of the plumular meristem in the seed embryo. In each cross the volume of the plumular meristem of the hybrid is intermediate between those of the parents. This intermediate position is maintained throughout the greater part of development. The changes in volume during the period in which the seedlings were grown on damp paper (the entries at 2, 4, and 6 days) are probably not significant because of the absence of any food supply other than that stored in the seed. There is, however, a marked change in meristem volume during the course of later development. As growth takes place, the volume of the apical meristem increases, reaching a maximum late in the grand period of growth and then falling off sharply to a relatively constant size. The point at which the decrease in volume takes place corresponds in most types with the falling off of the growth rate (Whaley, 1939).

The rather large variation within each group in the values obtained is no doubt due largely to the amount of error introduced by the involved method used. It is possible, however, that some of it may be due to differences in the point in the plastochron at which the samples were taken. That is, there may be a considerable difference in the volume of any given meristem just before and just after production of a leaf primordium, and no attempt was made to determine the length of a plastochron or to synchronize the intervals between samplings to this length.

Cross I (Lycopersicon esculentum  $\times$  L. pimpinellifolium).—The differences in the developmental behavior of the meristem of the parents and the hybrid of this cross suggest a relation of meristem volume changes to heterosis. A notable variance between the hybrid and its parents is that the decrease following the attainment of a maximum meristem volume comes later in the hybrid than in either parent. It occurs about two weeks later than in parent A and a week later than in parent B, which it resembles most in growth. This change corresponds closely to the points at which growth begins to slow down in all three types. There might, therefore, appear to be a correlation between the length of time during which the meristem remains large and the duration of growth, and thus the appearance of heterosis.

Cross II (Lycopersicon esculentum var. "Chinaman" × L. racemigerum).—The behavior of meristem volume here differs in two ways from that in cross I, although here, too, the hybrid is intermediate between its parents as to plumular meristem size. First, the apical meristem is larger throughout development in both C and D and their hybrid

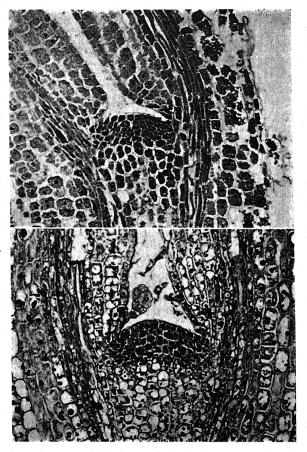


Fig. 14-15.—Fig. 14 (above). Photomicrograph of plumular meristem of parent A.  $\times 220$ .—Fig. 15. Photomicrograph of the apical meristem of a 7-day-old seedling of parent A.  $\times 220$ .

TARTE 1

	Α.		В		$_{ m A} \times$	В	c		D		$C \times 1$	D
	Meristem	Cell	Meristem	Cell	Meristem	Cell	Meristem	Cell	Meristem	Cell	Meristem	Cell
Days	volume, $\mu^3$	no.	volume, $\mu^3$	no.	volume, $\mu^3$	no.	volume, $\mu^3$	no.	volume, $\mu^3$	no.	volume, $\mu^3$	no.
0	1,482,000	659	249,000	230	853,000	609	1,246,000	937	506,000	363	886,000	791
2	1,121,000	704	290,000	334	1,029,000	832	1,257,000	1,091	651,000	777	844,000	1,035
4	745,000	550	352,000	500	544,000	484	1,438,000	2,025	604,000	772	670,000	1,012
6	903,000	986	325,000	503	623,000	877						
7	1,128,000	948	230,000	246	1,073,000	891	1,489,000	1,884	498,000	759	1,208,000	1,157
14	1,069,000	1,084	296,000	425	548,000	492	2,043,000	3,921	822,000	1,398	1,396,000	1,781
21	872,000	956	421,000	687	556,000	524	1,380,000	2,993	552,000	1,080	1,457,000	2,162
28	1,211,000	1,467	474,000	801	498,000	509	1,289,000	3,011	930,000	2,220	1,728,000	3,456
35	1,490,000	2,072	483,000	866	664,000	758	2,574,000	6,500	932,000	2,295	1,271,000	2,604
42	1,933,000	3,710	317,000	613	984,000	1,305	2,395,000	6,526	503,000	1,393	1,582,000	4,533
49	1,634,000	3,376	512,000	1,205	742,000	1,158	1,582,000	4,407	696,000	2,017	1,144,000	3,553
56	1,248,000	2,978	609,000	1,538	1,014,000	2,375	1,920,000	5,714	1,203,000	3,613	1,394,000	4,124
49	1,196,000	2,190	948,000	1,675	1,017,000	1,532	2,291,000	6,942	1,125,000	3,280	1,399,000	4,201
56	1,837,000	3,492	859,000	1,624	1,514,000	2,619	2,205,000	6,682	1,059,000	3,070	1,495,000	4,410
63	1,806,000	4,058	1,186,000	2,382	1,621,000	2,947	2,891,000	9,034	1,408,000	4,386	1,680,000	5,490
70	991,000	2,252	1,218,000	2,914	1,514,000	3,174	2,218,000	7,248	1,305,000	4,321	1,590,000	5,262
77	1,021,000	2,466	622,000	1,902	1,480,000	3,682	2,305,000	7,484	1,221,000	4,003	1,025,000	3,687
84	835,000	1,915	773,000	2,378	973,000	2,788	2,004,000	6,592	1,123,000	3,846	930,000	3,110
91	826,000	1,864	592,000	1,726	854,000	3,247	1,633,000	5,337	1,110,000	3,535	1,195,000	3,879
98	842,000	2,094	543,000	1,588	827,000	2,902	1,763,000	5,976	1,343,000	4,663	1,059,000	3,449
105	807,000	2,002	649,000	1,967	746,000	2,645	1,959,000	6,279	1,407,000	4,644	814,000	2,907

TABLE 2.

		A			В		A	$\mathbf{A} \times \mathbf{B}$	
Days	Cell volume, $\mu^3$	Nuclear volume, μ <sup>3</sup>	C/N ratio	Cell volume, $\mu^3$	Nuclear volume, μ <sup>3</sup>	C/N ratio	Cell volume, μ <sup>3</sup>	Nuclear volume, μ <sup>3</sup>	C/N ratio
0	2,248±139ª	101±5	22.2	1,082±91	66±1	16.4	1,400±106	90±5	15.5
. 2	$1,592\pm129$	$107 \pm 6$	14.9	$867 \pm 69$	$81\pm4$	10.7	$1,236 \pm 57$	$103 \pm 5$	12.1
4	$1,354 \pm 67$	124±5	10.9	$704 \pm 19$	$102 \pm 3$	6.9	$1,122 \pm 55$	$102 \pm 4$	11.0
6	$916 \pm 71$	$87 \pm 4$	10.5	$646 \pm 26$	$56 \pm 2$	11.5	711±51	$93 \pm 3$	7.6
7	$1,190\pm65$	112±7	10.6	934±30	$80 \pm 6$	11.7	$1,204 \pm 92$	$145 \pm 8$	8.3
14	$986 \pm 48$	$109 \pm 5$	9.0	$697 \pm 28$	$76 \pm 6$	9.2	$1,114\pm49$	$135 \pm 6$	8.3
21	912±58	$106 \pm 4$	8.6	$613 \pm 24$	$70 \pm 1$	8.8	$1,062 \pm 63$	$132 \pm 5$	8.
28	$825 \pm 49$	$98 \pm 3$	8.4	$592 \pm 25$	71±2	8.3	$978 \pm 43$	$124 \pm 5$	7.
35	$719 \pm 25$	$95 \pm 5$	7.6	$558 \pm 25$	$71 \pm 3$	7.8	$876 \pm 28$	$110 \pm 5$	7.
42	$521 \pm 32$	$79 \pm 4$	6.6	517±22	$66 \pm 4$	7.8	$754 \pm 33$	$103 \pm 7$	7.
49	$484 \pm 16$	$68 \pm 3$	7.1	$425 \pm 20$	58±2	7.3	$641 \pm 24$	$95 \pm 4$	6.
56	419±25	60±2	7.0	$396 \pm 16$	$59 \pm 3$	6.7	$427 \pm 22$	$85 \pm 5$	5.
49	$546 \pm 32$	83±3	6.6	566±21	$92 \pm 2$	6.2	$664 \pm 33$	$119 \pm 6$	5.0
56	$526 \pm 36$	$78 \pm 3$	6.7	529±23	$92 \pm 4$	5.8	$578 \pm 32$	$105 \pm 2$	5.
63	$445 \pm 23$	$73 \pm 2$	6.1	$498 \pm 20$	85±2	5.8	$550 \pm 34$	$99 \pm 3$	5.
70	$440 \pm 20$	$70 \pm 2$	6.1	418±15	$82 \pm 2$	5.1	$477 \pm 21$	$90 \pm 4$	5.
77	414±13	66±2	6.3	$327 \pm 25$	$72 \pm 2$	4.5	$402 \pm 20$	$80 \pm 5$	5.
84	$436 \pm 14$	58±2	7.5	$325 \pm 11$	75±2	4.3	$349 \pm 12$	$67 \pm 3$	5.
91	$443 \pm 16$	62±2	7.1	$343 \pm 15$	$76 \pm 2$	4.5	$263 \pm 10$	$57\pm1$	4.
98	$402 \pm 14$	58±2	6.9	$342 \pm 25$	$73 \pm 3$	4.7	$285 \pm 12$	$52\pm1$	5.
105	$403 \pm 11$	68±3	5.9	$330 \pm 16$	$70 \pm 2$	4.7	282±9	$54 \pm 2$	5.

a Standard error.

than it is in A, B, or the  $A \times B$  hybrid. Second, there is not the marked decrease in volume during the final stages of growth which was apparent in cross I. C and  $C \times D$  may show a slight decrease, but D shows none at all. This D race differs mark-

edly from any of the others in its behavior during the final growth stages (Whaley, 1939), slackening in its growth many days later than either C or  $C \times D$ . In this cross there does not seem to be any definite relation, such as was found in cross I, be-

TABLE 2. Continued.

		С			D		(	$C \times D$	
Days	Cell volume, $\mu^3$	Nuclear volume, μ <sup>3</sup>	C/N ratio	Cell volume, μ <sup>3</sup>	Nuclear volume, μ <sup>3</sup>	C/N ratio	Cell volume, μ <sup>3</sup>	Nuclear volume, $\mu^3$	C/N ratio
0	1,331±69	103±5	12.9	1,394±80	69±2	20.2	1,120±55	99±3	11.3
2	$1,152\pm111$	101±3	11.4	$838 \pm 32$	$92 \pm 4$	9.1	$815 \pm 41$	$81 \pm 4$	10.0
4	$710 \pm 49$	$94 \pm 3$	7.6	$782 \pm 42$	$75 \pm 3$	10.4	$662 \pm 31$	$73 \pm 3$	9.1
6	556±41	$90 \pm 2$	6.2	$678 \pm 49$	$86 \pm 2$	7.9	$613 \pm 21$	51±1	12.0
7	$790 \pm 37$	95±3	8.3	655±48	$92 \pm 2$	7.1	$1,044 \pm 42$	$123 \pm 9$	8.5
14	521±37	89±3	5.8	588±40	$87 \pm 2$	6.8	$784 \pm 38$	$93 \pm 3$	8.4
21	$461 \pm 20$	84±2	5.5	511±27	$81 \pm 3$	6.4	$674 \pm 33$	$90\pm 3$	7.5
28	428±25	84±3	5.1	419±19	$80 \pm 2$	5.2	500±21	$91 \pm 2$	5.5
35	$396 \pm 18$	77±2	5.1	$406 \pm 13$	$73 \pm 2$	5.6	488±21	$89 \pm 3$	5.5
42	$367 \pm 18$	71±2	5.2	361±15	65±2	5.6	$349 \pm 9$	76±2	4.6
49	$359 \pm 14$	74±2	4.8	345±9	60±2	5.8	$322 \pm 9$	$60 \pm 2$	5.4
56	336±7	68±2	4.9	$333 \pm 9$	$59 \pm 1$	5.6	$338 \pm 8$	58±1	5.8
49	$330 \pm 8$	$60 \pm 2$	5.5	$343 \pm 10$	$55 \pm 1$	6.2	$333 \pm 7$	42±1	7.9
56	$330 \pm 7$	$63 \pm 2$	5.2	$345 \pm 10$	$60\pm 2$	5.8	$339 \pm 4$	$41 \pm 1$	8.3
63	320±5	$65 \pm 2$	4.9	321±8	59±2	5.4	$306 \pm 9$	48±2	6.4
70	$306 \pm 7$	59±2	5.2	302±9	$56 \pm 3$	5.4	302±8	$33 \pm 1$	9.2
77	308±9	$56 \pm 1$	5.5	$305 \pm 6$	$48 \pm 2$	6.4	278±7	$26 \pm 1$	10.7
84	$304 \pm 6$	59±2	5.2	292±7	$51\pm1$	5.7	$299 \pm 7$	$28 \pm 2$	10.8
91	306±9	54±1	5.7	314±10	$56\pm2$	5.6	$308 \pm 9$	$31 \pm 2$	9.9
98	$295 \pm 6$	57±2	5.2	288±7	58±2	5.0	$307 \pm 9$	$25 \pm 1$	12.3
105	$312 \pm 6$	51±1	6.1	303±9	$52\pm2$	5.8	280±9	30±1	9.3

tween the development behavior of meristem volume and the appearance of hybrid vigor.

Cell volume.—An estimation of meristem cell size and its changes during development was undertaken in an attempt to ascertain whether heterosis might be manifest in some process reflected in cell size relations rather than in meristem size. The results are given in table 2 and figures 1 and 2. The most important fact to be observed is that in all cases, as development proceeds, cell size decreases. Actually no given cell decreases in size, but the apparent decrease results from a progressive reduction in the amount of cell growth between successive divisions. This diminution of cell size proceeds until about the time the growth curve begins to flatten off. At this point a minimum level is reached and maintained.

Cross I.—In this cross the plumular meristem cell size of the hybrid is intermediate between those of its parents. Nevertheless, there are two marked developmental differences between the hybrid and the pure types. Diminution of the cell size takes place less rapidly in the hybrid. Figure 1 shows this fact clearly, for the slope of the hybrid curve is much less steep than those of the parents. As a result, throughout early development the cells of the hybrid meristem are larger than those of its parents. A second difference lies in the fact that the minimal size, which is reached at maturity in each type, is smaller in the hybrid than in its parents.

Cross II.—This cross presents the same picture as the previous one, except for two facts. First, the plumular meristem cell size of the hybrid is smaller than that of either parent. Here again size decreases much more slowly in the hybrid, so that, despite its initially smaller cells, the hybrid cells are larger throughout most of development. Second, as compared with cross I, meristem cell size in all types here decreases much more rapidly (figure 2). This difference is correlated with a difference in general growth rates (Whaley, 1939, fig. 1, 2, 3 and 4, 5, 6). There does not appear to be much difference between the final minimal level reached by the hybrid and the pure types in this instance (table 2). Perhaps for this reason the increase of the hybrid over its parents during the final stages of growth is much less noticeable here than in cross I.

In both crosses there is a relation between the rate of change in meristem cell size during development and the degree of heterosis shown, diminution of cell size proceeding much less rapidly in the hybrids than in the pure types.

Nuclear volume.—Nuclear volume of meristem cells in parents and hybrids was also compared (table 2, fig. 3, 4). In both crosses the nucleus of the hybrid is intermediate in size between those of the pure types in the seed. In the initial stages of growth the nucleus behaves differently from the cell in that its volume increases for a short time either during or immediately after germination. The apparent absence of any increase in type C may be due to an error of measurement, or the increase may be followed by a decrease of equal magnitude during the

first seven days. At any rate, after seven days five of the six types have nuclei larger than those in their respective seed embryos, and the hybrid is in each instance markedly larger in this respect than either parent. Following this initial increase, the nucleus decreases in size during development in much the same manner as does the cell. Relatively, however, it decreases at a much less rapid pace. In both crosses a minimum nuclear size is reached at the same time that the cells reach their minimum size. As with the cells, this final size is lower in each of the hybrids than in the pure types.

Cell number.—As seen in table 1, the hybrid does not have a larger number of meristem cells than the parents in either cross, except, as pointed out above, in the final stages of growth. The behavior of cell number during development is, of course, a function of the relation between meristem volume and cell volume. Generally, cell number increases during development, with a slight decrease attending the change in both cell and meristem volumes at the attainment of maturity.

Discussion.—Size in an organism depends upon three variables: first, the size of the original cell or group of cells from which the organism grows; second, the rate of growth; and third, the duration of growth. The most obvious aspect of hybrid vigor is the attainment of greater size by the hybrid than by its parents. The first step toward an understanding of heterosis must be to determine which of the variables are concerned in the ultimate differences observable between the hybrids and the pure races. The results in the present investigation make it possible to evaluate the part played by each of these factors in the development of hybrid vigor in Lycopersicon. Since this paper is concerned mainly with post-embryonic growth, embryo size in the seed will be considered first. It is realized that any embryo size differences are in turn due to differences in rate or duration of growth between fertilization and the maturation of the seed.

Ashby (1930, 1932) postulated that the size of the embryo is the most important of the factors in relation to heterosis, and Luckwill (1937) found evidence to support him. The results presented here do not agree with those of Ashby and Luckwill. In cross I the hybrid embryo is larger than those of the parents, but in cross II, with almost as much heterosis, the hybrid embryo is intermediate in size between those of its parents. East (1936) pointed out that there are many other cases in which the embryos of vigorous hybrids are smaller than those of their parents.

There remains, however, the possibility, suggested by both Ashby and Luckwill, that the size of the actual meristematic tissue mass (the "meristematic capital"), and not the whole seed embryo, may be the important factor. If the actual mass of meristematic cells in the embryo is the determining factor in heterosis, then the measurement of the meristems at the tip of the stem or the root should indicate a volume difference in favor of the hybrid. The meristem of the shoot rather than that of the root was

chosen for measurement because from it originate the parts of the plant with which heterosis is known to be concerned, and because its developmental history may be more readily observed. Luckwill (1937) made some observations of shoot primordia in a hybrid tomato and its parental types, from which he concluded that the hybrid meristems were larger than those of the pure types. Luckwill's drawings, however, include differentiated tissue which must be many times as large as the primordia of which he speaks, and are without much value as a measure of the active meristematic region. Ashby and Luckwill were concerned with the size of the plumular meristem when they assumed the amount of "original meristematic capital" to be the immediate controlling factor in the development of hybrid vigor. Reference to table I shows clearly that there is no relation between the size of the plumular meristem and the presence of heterosis, since in both crosses the hybrid is intermediate between its parents.

There is still the further possibility that meristem size at some time during later development may be a deciding factor. At first glance the results from cross I might seem to lend support to such an idea, since, although during the greater part of development the hybrid meristem maintains its intermediate position, it retains its large size considerably longer than either of the pure types. The results from cross II make the acceptance of this idea impossible, for if there is actually a decrease in meristem size here (and in D and C X D this is not at all certain), the hybrid decreases earlier than the parental types. It is, therefore, clear that meristem volume, at least in so far as the conception of the meristem here presented is concerned, either in the seed embryo or during development, is not concerned in heterosis.

Size of the meristem seems to be correlated directly with size of the lateral determinate organs of the plant, as suggested by Sinnott (1921), rather than with total plant size. Evidence for this assumption is seen in the sizes of the meristems in each cross and also in the differences between the meristems of the plants in cross I and cross II. The cross II plants are all larger as to organ size than those of cross I and have correspondingly larger meristems. Sinnott has shown that for a time during development there is progressive increase in the size of the organs produced. If size of an organ is determined by size of the meristem from which it comes, then an increase in organ size during development should be paralleled by an increase in meristem size. The material here studied shows that this is the case.2 The problem of the inheritance of organ size in plants thus becomes one of the inheritance of meristem size.

The close relation between the size of the meristem and of the determinate organs which it produces, and the absence of a relation between heterosis and meristem size, explains the fact so often

<sup>2</sup> The decrease in meristem volume which sometimes accompanies the attainment of maturity may be the result of a greatly diminished rate of cell division or of a diversion of nutrient substances to the developing fruits. It is usually paralleled by a decrease in organ size.

observed that heterosis does not express itself in the size of these determinate organs.

Meristem volume is also related to the growth habit of the plants. The gene d, which results in the dwarf habit when present in the homozygous condition, seems to operate by increasing the size of the meristem. Large meristems produce thicker stems with less growth in length than those formed from smaller meristems. In both crosses the Dd hybrids have a tall growth habit, but in each case, because of heterosis, they are both taller and heavier than the tall parents. This size increase, which is due to greater growth and a larger number of plant parts, is not accompanied by an increase in meristem size. The dwarf parent in each instance has the largest meristem. Meristem volume is thus related to gene D-d and not to the presence of heterosis.

The second factor is the rate of growth. Ashby (1930, 1932, 1937) has presented evidence to prove that at least during the period of exponential growth there is no difference in rate of weight increase between the hybrid and the faster-growing of its two parents. The author's results are in agreement with Ashby's in so far as there does not appear to be any significant difference during this period. In the very early seedling period, however, the hybrids grow much faster than the pure types. Failing to find any difference in the rates of exponential growth, Ashby supposed the advantage which the hybrids possessed to be the result of greater embryo size—an assumption in which he apparently was not justified, at least in so far as tomatoes are concerned. The size advantage of the hybrid may be the result of more growth in the very early seedling stages. Sprague (1936) has also found such to be the case in maize. If, in addition, the hybrid embryo is larger than those of the parents, as in cross I, a still greater hybrid superiority may result.

The third factor, duration of growth, does not seem to be primarily concerned in the development of hybrid vigor. Ashby has shown that in both maize and tomatoes the hybrid does not exceed its faster-growing parent as to length of the period of exponential growth. The data presented here support this conclusion, but indicate that while growth may begin to slacken in the hybrid at the same time that it does in the parents, it usually falls off less rapidly in the hybrid, with the result that there is more growth during later stages. These later differences are accentuated if the whole plant rather than the vegetative parts alone are considered.

Hybrid vigor in the *Lycopersicon* crosses reported here is thus the result of more rapid growth of the hybrid during the early stages, augmented in the final stages by less rapid slackening of growth. It seems probable, therefore, that the operation of heterosis depends upon more rapid growth at some time in development. It may take place during the development of the embryo, in the early seedling stages, in the final fruiting stages, or in any combination of these. It seems to be absent or restricted during the grand period of growth.

Although volume of the meristem does not seem to be in any way concerned in hybrid vigor, a study of developmental changes in the meristem does furnish some very important clues as to the manifestation of heterosis. The first of these lies in the behavior of cell size.3 Whereas differences in cell size at the meristem at any given time are not one of the manifestations of heterosis, the behavior of cell size during development is evidently closely related to the duration of growth and thus to final size differences. Developmental changes in the size of apical meristem cells have been briefly reported in grass embryos by Rössler (1928) and in soybeans by Murneek and Gomez (1936). These results, and the much more extensive ones here presented, indicate that there is a progressive diminution in cell size in the apical meristem of an annual plant from germination until the end of the grand period of growth. At about this point, which marks the end of active growth and the onset of maturity, a minimal cell size is attained which thereafter remains essentially constant. This fact is of rather general significance for an understanding of growth, and a comparison of pure races and hybrids in this regard is, therefore, important in an analysis of heterosis. The diminution in cell size during development evidently results from failure of the daughter cells to equal the size of the mother cell before they divide again. The balance between the rate of division and rate of enlargement determines the relative rate of cell size change. It is evident from a comparison of the slopes of the curves (fig. 1, 2) that during the early stages of growth the decrease in cell size until uniform minimum size is attained takes place less rapidly in the hybrids than in either of the pure types. There are two possible reasons; cell division may be less rapid, or rate of intake of materials, and thus of protoplasmic synthesis, may be more rapid. The first possibility is unlikely, because the hybrid does not grow more slowly than either parent. Thus the difference between hybrids and pure types seems fundamentally to be one of metabolism. In both, cell size at the meristem is gradually falling as maturity approaches, each generation of daughter cells failing to reach the size of their mother cells; but in the hybrids the more vigorous production of new synthetic material markedly retards this process, deferring the attainment of minimal cell size and resulting in more growth, and thus in the result which we recognize as heterosis.

Nuclear volume seems also to be of significance in the distinction between hybrid and pure types. Bindloss (1938) studied nuclear size in the plumular meristems of inbred and hybrid maize. In one cross the plumular meristem nuclei of the hybrid were intermediate in size when compared with those of the parents, while in another the hybrid nuclei were

<sup>3</sup> Kostoff and Arutiunova (1936), Malinowski (1935), and others have presented evidence to show that mature cell size is not a factor in heterosis. In the present experiment neither hybrid possessed significantly larger cells than the parents when petiole parenchyma cells were used as an index. Accordingly, no further attention has been given to cell size, other than in the meristem.

larger. In both Lycopersicon crosses reported here the hybrid has plumular meristem nuclei intermediate in size between those of the parental types. Here, too, as with the cells, absolute size in the plumular meristem offers no suggestion of any relation to heterosis. The key to the situation lies in the developmental behavior of the nuclei. Early growth is accompanied by a great increase in nuclear size. The greater increase in the hybrids than in the pure types gives them a nuclear size advantage which is then maintained throughout most of development. The hybrids differ from their parents further in that the minimal size to which their nuclei finally decrease is much smaller than that for the pure races. For some reason the hybrid nuclei possess the ability to divide at a smaller size than those of the parental types. These nuclear differences, like those of the cells, seem to be indicative of some physiological difference between the hybrid and the pure types.

Changes in cell and nuclear size during development make it desirable to calculate the cell/nucleus ratio in order to determine whether the absolute size of the cells and nuclei or the relationship of one to the other is important. Sinnott and Trombetta (1936) and Trombetta (1939) have shown a definite relationship to exist between rate of increase in size of cell and nucleus. They found nuclear volume to increase generally about two-thirds as fast as cell volume. As a result, the ratio of cell size to nuclear size is much greater in larger cells than in smaller ones. This same relation obtains in the material studied here (table 2, fig. 5-15), although the course of development is from larger to smaller cells rather than vice versa. Sinnott (1939) has shown that in simple determinate growth, as in the development of fruits, size of the cells increases until finally a point is reached at which cell division ceases. In the indeterminate growth of the meristem the reverse takes place, for here the cells become progressively smaller until finally they reach a minimal size. Cessation of cell division and thus of meristematic activity may be in part the result of changes in the relation of cell to nucleus.

We may conclude, therefore, that the direct causes of heterosis lie primarily in physiological differences between hybrids and pure types. The presence of these differences is apparent in the more rapid growth of the hybrids at some time during development. This more rapid growth need not be confined, as Ashby and Luckwill believe, to the period of development of the embryo. These metabolic differences may also be demonstrated by a comparative study of the meristems of hybrids and their parents, for the synthesis of protoplasm, as indicated by size changes in meristem cells and nuclei, is more rapid in the hybrids. A knowledge of conditions at the meristem, therefore, contributes markedly to an understanding of heterosis, although meristem volume does not seem to be directly concerned.

#### SUMMARY

A study was made of certain developmental changes in the volume, cell size, and nuclear size in

the apical meristems of two Lycopersicon species crosses showing heterosis.

No relation was found, either in the embryo or during development, between the volume of the apical meristem and heterosis.

Size of the meristem is related to the size of the determinate organs developed from it. These organs are unaffected by heterosis.

Cell and nuclear size in the meristem was found to

decrease during development, but much less rapidly in the hybrids than in the pure types. The attainment of a minimal cell and nuclear size is associated with the onset of maturity. These differences seem to be due to a fundamental metabolic difference between pure types and hybrids.

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# A REVISION OF THE SUBGENUS DIHOLCOS OF THE GENUS ASTRAGALUS <sup>1</sup>

### C. L. Porter

Because of the increasing interest in the seleniferous plants, many of which are Astragali, and the attendant need for a critical examination of the many species of that group, an intensive study of the genus Astragalus in its broad sense has been started. This paper deals with only one of the subgenera of the genus. Other subgenera will be similarly treated as the work on them is completed.

The first question naturally arising is that of whether to treat the group as a genus containing several well-defined subgenera, or to maintain these major divisions of the group as distinct genera. There are good reasons for either treatment, but the former method is here regarded as more desirable for the following reasons: (1) Although there are undoubtedly good generic differences in the group, these are mostly based on fruit characters alone and are not evident in immature specimens. (2) Those divisions of the group which are distinguishable on

<sup>1</sup> Received for publication May 29, 1939. Contributions from the Department of Botany and the Rocky Mountain Herbarium of the University of Wyoming, No. 174. vegetative characters (such as the spiny leaves in the subgenus Kentrophyta and the trifoliate leaves in the subgenus Orophaca) are certainly as closely related to other members of the group as are those which differ chiefly in fruit characters. (3) Field workers find it desirable to be able to give a generic name to a plant even though it lacks the maturity necessary for a generic diagnosis which is based on fruit characters. (4) Herbarium workers find it desirable to be able to place a specimen in a genus at least, even though it may be immature, and thus file it in the cases with its near relatives. If the genus is not known, it must be placed in the enquirendi for the whole family. Furthermore, the segregates from such a group, when treated as genera, would be filed in an herbarium alphabetically, and thus groups which are more closely related to each other than to other groups in the family might be widely separated in an herbarium merely because they have been designated by generic names which start with various letters of the alphabet.

In the course of this study the writer has seen either type or topotype material of all species and

synonyms with the single exception of that of Astragalus bisulcatus, a very well-known species whose type is not available in this country. In addition, many of the specimens annotated by Dr. Rydberg and by Mr. Marcus Jones have been studied. The valuable collection of Mr. George E. Osterhout, a close friend of Mr. Jones, which contains most of the plants distributed by him, has been studied, together with that of the Rocky Mountain Herbarium. The writer has also studied and named many sheets of specimens from the Soil Conservation Service through the assistance of Mr. L. N. Goodding, botanist for that branch with headquarters in Albuquerque, New Mexico. Many specimens have been studied from the collection of Prof. O. A. Beath, Research Chemist at the University of Wyoming, who has collected extensively wherever Astragali are likely to be seleniferous. The writer also has seen most of the species growing in the field in Wyoming, Utah, Colorado, and New Mexico, and has collected numerous specimens of the species encountered. Because of the excellent representation of specimens seen as indicated above, it was not deemed essential that the collections of all the large herbaria be stud-

ied, except as to the borrowing of types.

The writer is indebted to the following for assistance in the preparation of this treatment: the herbarium of Pomona College, California, for the loan of the type of Astragalus oocalycis; the herbarium of the New York Botanical Garden for the loan of the type of Astragalus haydenioides (Diholcos micranthus); Prof. O. A. Beath and Mr. Carl Gilbert, research chemists at the University of Wyoming, for helpful data on distribution and variation

of several species; Mr. L. N. Goodding, botanist for the Soil Conservation Service, for specimens collected particularly in the southwest; and to Dr. Aven Nelson, curator of the Rocky Mountain Herbarium, for helpful advice and suggestions.

ASTRAGALUS SUBGENUS DIHOLCOS RYDB.—Plants perennial, often caespitose, 2-10 dm. high, with erect or sometimes decumbent stems from woody roots. Leaves pinnately compound, with about 10 (8-15) pairs of linearoblong to elliptic leaflets and free stipules. Flowers in dense or loose racemes or spikes, white, cream-colored, or purple, horizontally spreading to reflexed at maturity, 7-15 mm. long. Calyx campanulate or urceolate, gibbous at the base above, with narrowly lanceolate to subulate teeth. Corolla with oblanceolate to narrowly obovate, retuse, clawless banner; the wings with an acute basal auricle, their blades about equaling the banner, obliquely oblanceolate; the blades of the keel broader and broadly lunate. Pods stipitate, coriaceous, the body 8-20 mm. long. oblong, straight, 1-celled, with no intrusion of either suture, 2-grooved ventrally and convex dorsally, glabrous or strigose and smooth or cross-reticulate.

Type species: Astragalus bisulcatus A. Gray.

### Key to the species

Calyx campanulate, not inflated, strigose, leaflets ovate

Body of pod 11 mm. long or more.....1. A. bisulcatus Body of pod 10 mm. long or less

linear......4. A. oocalycis

Since the above tabulation provides a fairly accurate description of the species, the discussion to follow will be limited to matters of variability and

Astragalus subgenus Diholcos	bisulcatus	Haydenianus	hay denioides	oocalycis
Pods glabrous or strigose	gors	s	s	g (when young)
Length of body of pod (mm.)	12-20	8-10	8-9	a
Length of stipe of pod (mm.)	3-4	3-5	3-4	a
Pod cross-reticulate	No	Yes	No	Yes
Color of flowers	Purple, rarely white	White, with pur- ple-tipped keel	Cream	White or cream, with purple- tipped keel
Length of corolla (mm.)	1220	8-12	6-7	15-16
Calyx tube inflated in age	No	No	No	Yes
Length of calvx tube (mm.)	4-6	3	2-2.5	8
Length of calyx teeth (mm.)	2-6	1-2	1-1.5	2-3
Pubescence on calyx	Strigose	Strigose	Strigose	Shaggy-villous
Shape of leaflets	Oblong to oval	Oblong to oval	Linear or lance- oblong	Linear or oblong
Length over width of leaflets (mm.)	10-25	7-25	7-20	10-40
	4-10	3-5	2-4	1-3
Number of leaflets	15-27	15-23	17-25	15-19
Range	Manitoba and Alberta to Ne- braska, Okla- homa, Idaho, Colorado, Wyoming, and northern New	Utah, Wyoming, Nevada, Colo- rado, and New Mexico	Colorado	Southern Colo- rado and northern New Mexico

<sup>&</sup>lt;sup>a</sup> Mature fruit unknown.

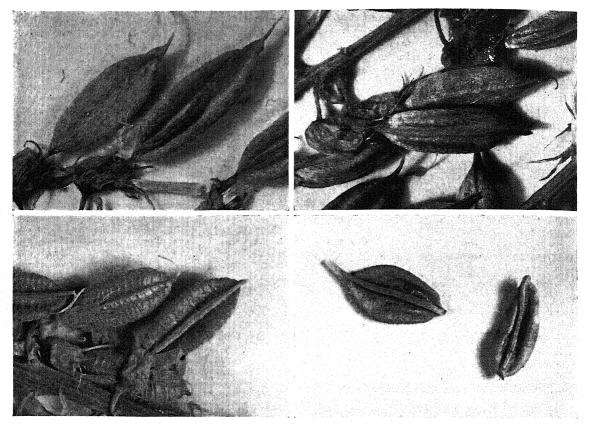


Fig. 1-4.—Fig. 1 (upper left). Astragalus bisulcatus (Hook.) Gray. A. Nelson No. 11506, Cimmaron, New Mexico. Pods.  $\times 4.2$ .—Fig. 2 (lower left). Astragalus Haydenianus A. Gray. Osterhout No. 4951, Hayden, Routt County, Colorado. Pods.  $\times 4.2$ .—Fig. 3 (upper right). Astragalus haydenioides Porter n. nov. From the type specimen. Pods.  $\times 4.2$ .—Fig. 4 (lower right). Astragalus oocalycis Jones. From the type specimen. Immature pods.  $\times 4.2$ .

synonymy, together with the citation of a few representative specimens that have been distributed to various herbaria.

1. ASTRAGALUS BISULCATUS (Hook.) Gray, Pacific R. R. Rep. 12: 42, 1860. Phaca bisulcata Hook., Fl. Bor. Am. 1: 145, 1831. Tragacantha bisulcata Kuntze, Rev. Gen. 943, 1891. Astragalus bisulcatus f. hedysariformis Gand., Bull. Soc. Fr. 48: XIV, 1902. Astragalus Haydenianus f. leiocarpa Gand., l. c. 48: XV, 1902. Astragalus bisulcatus f. decalvans Gand., l. c. 48: XV, 1902. Diholcos decalvans Rydb., Bull. Torr. Bot. Club 32: 664, 1906.

The campanulate or tubular calyx tube combined with the large flowers and fruit distinguishes this species from the others. The color of the flowers is usually a deep purple, but it may vary from that through pale lavenders to cream-colored or white. Furthermore, the pods are usually strigose, but the degree of this character may vary, and sometimes the pods are quite glabrous. The color of the corolla is not correlated with the strigosity or glabrousness of the pods, therefore there appears to be no basis for the recognition of Gandoger's forma decalvans in any category.

Type—collected by Drummond, from the plains of the Saskatchewan, Canada, in the Kew Herbarium.

CITED SPECIMENS--COLORADO, Denver, Clokey 3805; near Pueblo, Rydberg & Vreeland 5964; base of Mesa Verde, A. Nelson 10438; Hayden, Osterhout 5090; Montrose, Payson 1035. Manitoba, Oak River Valley, Burman in 1897. Montana, Poplar, Larsen 143; Livingston, Blankenship 668. New Mexico, Las Vegas, Cockerell 27 and Aven & Ruth Nelson 2203; Cimmaron, A. Nelson 11506. North DAKOTA, Marmarth, Moyer 508; Devils Lake, Joyce Peterson in 1934; near Minot, Waldron 1841. Sas-KATCHEWAN, N. Battleford, June 18, 1912 (no collector or number given, but this represents a collection from near the type locality). South Dakota, Hermosa, Rydberg 629; Sturgis-Bear Butte, Mc-Intosh 281; Cave Hills, Over 17566. WYOMING, Medicine Bow River, A. Nelson 4088; Laramie, A. Nelson 10591; Wamsutter, A. Nelson 10724; South Sybille, A. Nelson 7380; Moorcroft, A. Nelson 2242; between Sheridan and Buffalo, Tweedy 3160; Sheep Mountain, Goodding 2110; Rock River, Macbride 2769; Lusk, Osterhout 7875.

2. ASTRAGALUS HAYDENIANUS A. Gray, in Bull. U. S. Geol. and Geogr. Surv. Terr. 2: 235, 1876.

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This species is distinguishable by the combined characters of small flowers and small, conspicuously cross-reticulate pods. The shape of the pods, whether acute or blunt, the length of the stipe, and the thickness of the leaflets are variable characters and do not justify specific or varietal distinctions.

Type—collected by H. N. Patterson, on Grand River, Middle Park, in the U. S. National Herbarium.

CITED SPECIMENS—COLORADO, Mancos, Jones 101 and Osterhout 1918; Montrose, Baker 11; Pagosa Springs, Bethel, Willey, & Clokey 4194 and Baker 412; Ridgway, Payson 2313; Muddy Pass, Aven and Ruth Nelson 134; 10 miles south of Kremmling, Goodding E488; Hayden, Osterhout 4951; Walcott, Osterhout 2661. New Mexico, Dulce, Castetter 2069; Nutrias, Wooton 2652; Cuba, Goodding E375. Utah, Price, Jones 5466; Emma's Park, Jones 5590g; north of Glendale, Aven and Ruth Nelson 2897; 2 miles north of Monticello, Porter 1791. Wyoming, North Vermillion Creek, A. Nelson 3786; Leucite Hills, Merrill & Wilcox 490.

3. ASTRAGALUS HAYDENIOIDES n. nov. Diholcos micranthus Rydb., Bull. Torr. Bot. Club 34: 420, 1907. Not Astragalus micranthus Nutt., Jour. Acad. Phila. 2: 122, 1821. Not Astragalus micranthus Desv., 1814.

The citation for *Diholcos micranthus* Rydb. is given by Rydberg in the North American Flora as his Flora of the Rocky Mountains. This is an error.

The preoccupation of the combination Astragalus micranthus necessitates a new name for this species and the above name was chosen because of the striking resemblance of this species to A. Haydenianus from which it differs chiefly in lacking the cross-reticulations on the pod possessed by that species.

TYPE—collected by C. L. Shear, No. 3569, at La Veta, Colorado, in the herbarium of the New York Botanical Garden.

CITED SPECIMENS—COLORADO, below Cerro Summit, Montrose County, Osterhout 6565.

4. ASTRAGALUS OOCALYCIS Jones, Contr. West. Bot. 8: 10, 1898. Cnemidophacos urceolatus Rydb., Fl. Rocky Mts. 502, 1917. Astragalus urceolatus Green, MS, Rydb. Fl. Rocky Mts. 503, as synonym, 1917. Diholcos oocalycis Rydb., N. Am. Flora 24: V, 282, 1929.

The urceolate, shaggy-villous calyx tube which is inflated in age renders this species very distinct from all others of the group. Unfortunately mature fruits have apparently never been collected, the best obtainable being two very immature pods on the type sheet which were photographed and are reproduced herewith. This spring the writer visited the type locality to attempt to collect more mature specimens, but not a single plant of the species could be found in the bottom lands at Aztec, New Mexico, the location described by Wooton. Much of that area has been cultivated since his time and perhaps the plants have been exterminated locally. Other localities in the region likewise failed to produce a specimen.

The vegetative aspect of the species, as pointed out by Jones in his Revision of North American Astragali, resembles that of A. racemosus. The writer does not agree with Jones, however, that this species is possibly a freak. On the contrary, it is one of the most excellent and consistently distinct species in the entire genus.

Type—collected by Wooton, in bottom lands, Aztec, New Mexico, in the Pomona College Herba-

CITED SPECIMENS—COLORADO, west of Pagosa Springs, Osterhout 6715; Bayfield, Bethel in 1917; Arboles, Baker 424.

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# EFFECT OF PHOTOPERIOD AND TEMPERATURE UPON THE GROWTH OF SEEDLINGS AND CUTTINGS 1

B. Esther Struckmeyer and R. H. Roberts

SEEDLINGS AND clons of several species that were grown in different environments for anatomical investigations2 made several interesting responses. Two of these were: (1) a conspicuous difference in the degree of variability of growth within a population for the different species of plants in the various environmental locations, and (2) the unlike reaction of seedlings and cuttings to photoperiod.

Material for a comparison of the response of the seedlings and clons to photoperiod was obtained by collecting seed from a single plant and then using this same plant as a source for cuttings. Several species of plants were grown, but emphasis will be limited to three: Centaurea cyanus L. (cornflower), Antirrhinum majus L. (snapdragon), and Petunia hybrida Vilm. When the seedlings and the plants from cuttings were two to three inches tall, the plants were placed in the different temperature locations with different lengths of day in each. Cool, medium, and warm houses with night temperatures of 55°, 65°, and 75°F., respectively, were maintained.

RESULTS AND DISCUSSION.—Because of the difference in the genetic composition of seedlings it is generally anticipated that a seedling population will show a greater amount of variability in growth than will a clon. The present results demonstrate that photoperiod and temperature also exert an influence upon the variability in growth of both clons and seedlings (fig. 1). This is shown by plants of a clon of Petunia that grew more uniformly in the short days at a cool temperature than in the long days at the same temperature. A similar effect of environment upon variability of growth was apparent with cuttings of alfalfa, Antirrhinum, Begonia semperflorens, Centaurea, Chrysanthemum, potato, Rudbeckia, and Salvia. The different species do not respond alike to a given environment, for one species may show a uniform growth, and another species may be variable under the same treatment. For example, Rudbeckia grew more uniformly in warm, long days, whereas Petunia plants were variable under this same treatment.

While seedlings show considerable variability in maturity and size of plants in some of the environments, they remain strikingly uniform in others. Seedling plants of Mathiola incana (stock) grew and flowered uniformly in cool long days, while they made a very variable growth in the medium temperature and short days (fig. 1B). A similar condition of

<sup>1</sup> Received for publication July 13, 1939.

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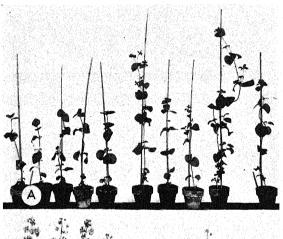
Supported in part by a grant from the Wisconsin Alumni Research Foundation.

<sup>2</sup> The writers herewith express their appreciation for the assistance of Mr. Leonard Langord in the growing of many of the plants used in this investigation.

uniformity and variability of growth, as influenced by environment, was noted for seedlings of Centaurea, Chinese cabbage, corn, Oenothera, peas, penny cress, pepper, Petunia, snapdragon, sorghum, and wild ground cherry.

It seems probable that the frequently reported "breaking up" of an apparently pure line of plants when grown for trial in an environment which is different from the one where the original selections were made is possibly the same type of phenomenon as shown in figure 1. This would indicate that the practice of growing new selections in different environments should probably be included in the technique of developing new strains of plants for breeding purposes.

It has already been pointed out that different varieties of a species show a wide range of adaptation



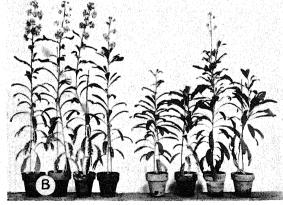


Fig. 1.-A. Petunia. Clons do not grow uniformly in all environments. The five plants at the left were grown in cool, long days. The five plants at the right were grown at a medium temperature with long days.—B. Stock. Seedlings show a relatively uniform growth in some environments. The plants at the left were grown at a cool temperature and long days. The plants at the right were grown at a medium temperature and short days.

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to a particular environment (Roberts and Struckmeyer, 1938a, 1938b). From the present experiments it appears that the environment may at times play an important rôle in affecting the variability or uniformity of the growth of a population of plants.

A comparison was made of the flowering responses of cuttings and seedlings to photoperiod and temperature. Seedling plants of Petunia flowered in long days before they did in short days (fig. 2A). On the other hand, plants propagated by cuttings came to flower at practically the same time in both short and long days. Because these cuttings were taken from flowering plants, the question arose as to whether cuttings from non-flowering plants would respond in a similar manner. The next procedure was to make cuttings from both flowering and nonflowering plants. When these and seedlings of a comparable stage of growth were grown out-of-doors in the summer of 1938, cuttings from the flowering plants blossomed in both the long and short days; those from the non-flowering plants budded in both lengths of day after the same period of time; and seedlings budded in the long days but remained

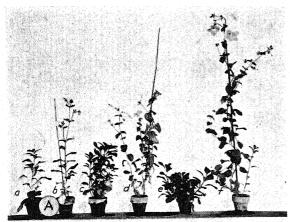




Fig. 2.—A. Petunia. Plants grown from seed. a, cool, short-day (budding); b, cool, long-day; c, medium temperature, short-day; d, medium temperature, long-day; e, warm, short-day; f, warm, long-day. Plants in short days remained vegetative except at a cool temperature, while all plants in the long days flowered.—B. Seedlings and cuttings. a, d, seedlings which remained vegetative in cool, short days and budded in cool, long days; b, e, cuttings from a non-flowering clon budded in both, cool, short and long days; c, f, cuttings from a flowering clon flowered at a cool temperature in both short and long days.

vegetative in the short days (fig. 2B). When this experiment was repeated in the fall and winter of 1938, similar differences in the responses of plants from different sources were noted.

Under the warmer night temperatures in the greenhouse during the summer, the different sets of plants gave similar flowering responses. The seedlings and both lots of cuttings were vegetative in short days and flowered in the long days (fig. 3).



Fig. 3. Seedlings and cuttings from a clon of Petunia. A, seedling; B, cutting from non-flowering clon; C, cutting from flowering clon; D, seedling; E, cutting from non-flowering clon; F, cutting from flowering clon. A, B, and C were in warm, short days, and D, E, and F were in warm, long days. The short-day plants were vegetative and the long-day plants flowered when kept warm.

A series of plants of snapdragon gave responses similar to those reported for *Petunia*. During the winter, cuttings from flowering plants flowered in all locations at about the same time, while seedlings flowered only in the long-days after a similar length of time. The plants grown out-of-doors in the summer responded differently to temperature. The seedlings remained vegetative for a considerable time, plants from non-flowering cuttings soon budded, and those from flowering cuttings quickly flowered.

Plants of *Centaurea* reacted like *Petunia* and snapdragon. Figure 4 shows the type of growth made by seedlings and cuttings of *Centaurea* in a cool location. Seedlings responded to photoperiod more strikingly than did cuttings. In a warmer location both seedlings and cuttings gave photoperiodic responses.

Since previous observations have shown consistent differences in the anatomy of flowering and nonflowering plants (Struckmeyer and Roberts, 1939; Wilton and Roberts, 1936; Wilton, 1938), samples of plants of Petunia were taken to determine whether the anatomical structure would throw any light upon the question of why the seedlings and the two lots of cuttings react differently to some combinations of photoperiod and temperature. Differences in the vascular anatomy of flowering and non-flowering stems as to the degree of cambial activity, the amount of phloem and xylem formation, and the cell sizes and maturity of the tissues have been repeatedly noted. As already reported for other regenerating shoots, the formation of new phloem as well as xylem below the regenerating node is associated with a vegetative shoot (Struckmeyer and Roberts, 1939). When the new shoot flowers rather quickly,

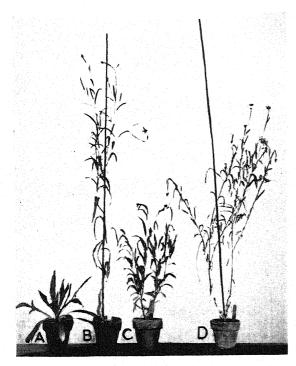


Fig. 4. Centaurea. A, seedling, short-day; B, seedling, long-day; C, cutting, short-day; D, cutting, long-day. The plants grown from seedlings remained vegetative in the short days and flowered in the long days. The cuttings on the other hand flowered in both lengths of day.

the ring of new tissue appears to be composed almost entirely of xylem cells.

An examination of sections of the original cuttings from non-flowering plants revealed the characteristic type of structure which is generally associated with the vegetative state, as shown by figure 6. The cuttings from flowering plants possess anatomical characteristics which are consistently found in flowering plants (fig. 8).

Figures 5 to 8 show cross-sections of stems of plants B and E, figure 3. In warm short days the cuttings from non-flowering plants remained vegetative. The original cutting still possessed an active cambial region with phloem and xylem cells in the process of differentiation (fig. 5). The new vegetative shoot from this cutting displayed an active cambium forming xylem and phloem cells (fig. 6). In warm long days the cuttings from non-flowering plants blossomed rather early. Observations of the cross-section of the stem of the original cutting shows that the cambial region is limited to only a few meristematic cells. Xylem but not phloem was formed as the stems increased in diameter. The cell walls of the tissues have become thickened. The pericycle fibers also have thicker walls in the flowering than in the vegetative stem. The new flowering shoot from this cutting presented anatomical features similar to those described for the original cutting from flowering plants (fig. 8).

Cuttings from flowering plants gave rise to vegetative shoots in a warm short-day environment (fig. 3). From observations of the cross-section of the original cutting it was found to have acquired vegetative characteristics as the cambial region had increased in width and activity, and new phloem and xylem tissues were being formed (fig. 9). The anatomical structure of the stems of the new shoots was like that described for the vegetative shoots of the non-flowering cuttings (see fig. 6). In the long days the cuttings from flowering plants gave rise to shoots that flowered. Both the old and new stems possessed anatomical characteristics of flowering stems (fig. 10 and like fig. 8).

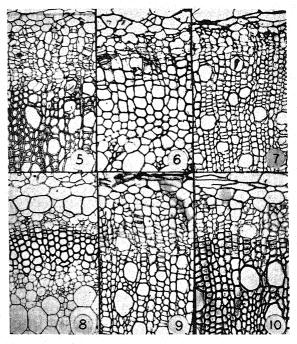


Fig. 5-10. Petunia.-Fig. 5. Cross-section of old nonflowering cutting which is producing vegetative shoots. This stem shows an active cambium and the differentiation of new xylem and phloem elements.-Fig. 6. Crosssection of new vegetative shoot which arose from the old cutting (fig. 5). The cambium is fairly active and the differentiation of new elements is in progress.-Fig. 7. Crosssection of old non-flowering cutting which gave rise to new shoots that flowered. The old cutting shows the cambium in a much less active condition than the vegetative cutting. The xylem and phloem cells have become mature, and few or no elements are in the process of differentiation.—Fig. 8. Cross-section of a new flowering shoot from cutting shown in figure 7. (Compare with figure 6.) The cambium is relatively inactive, and no new phloem cells are being formed.—Fig. 9. Cross-section of an old cutting which was taken from a flowering plant, but which gave rise to vegetative shoots in warm short days. The cutting has acquired the characteristics of vegetative stems. The cambium is active, and new vessels and phloem cells are being formed.-Fig. 10. Cross-section of cutting from a flowering plant which gave rise to flowering shoots in long days. (Compare with figure 9). The cambium is less active, and little vascular tissue is being formed.

When grown out-of-doors in the summer, the seed-lings and cuttings of *Petunia* did not give the same responses to photoperiod as the plants in the warm greenhouse (fig. 2B). Observations of sections of the cuttings from non-flowering plants and of the new shoots which were in an advanced stage of budding disclosed anatomical conditions like figures 7 and 8, which are typical of stems from flowering plants. The cuttings from flowering plants produced blossoms quickly in both long and short days. The anatomical structure of these stems and their new blossoming shoots was similar to those pictured for the plants in the warm long-day environment which remained in a flowering state (see figures 8 and 10).

In a warm environment photoperiod had a pronounced influence upon the growth of cuttings of Petunia. The anatomical structure and the type of growth of the cuttings from flowering plants was changed to the vegetative condition when grown in short days (fig. 3C). Seedlings and cuttings from non-flowering plants remained vegetative. Long days stimulated plants of all three sources to flower quickly (fig. 3D).

At a cooler temperature photoperiod had less effect, especially upon those plants grown in short

days.

The anatomical structure and type of growth of the cuttings from flowering plants continued unchanged, and plants from these cuttings blossomed earlier than plants from cuttings taken from nonflowering plants and also much earlier than seedlings.

The source of the plants—that is, whether they were seedlings or cuttings—determined to a large extent the kind and rate of response they made to

photoperiod.

#### SUMMARY

The degree of variability within a population of seedlings or of a clon was influenced by the environment. Both seedlings and clons were sometimes uniform and sometimes variable in growth, depending upon the environmental treatments to which they were exposed.

Cuttings of Antirrhinum, Centaurea, and Petunia responded differently to photoperiod and temperature than did the seedlings of these same genera.

The difference in response of cuttings from non-flowering plants and from flowering plants to environment was found to be associated with the differences in the anatomical structure of the original cuttings.

The flowering of seedlings was also found to be associated with the structure of the stem.

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# NOTES ON THE DISTRIBUTION AND ECOLOGY OF ANANAS AND PSEUDANANAS IN SOUTH AMERICA <sup>1</sup>

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ALTHOUGH PINEAPPLE, Ananas comosus (L.) Merrill, and its near allies have been long considered native to South America, and opinions have been expressed<sup>2</sup> concerning more definite localization, it is only recently that comprehensive field and herbarium evidence have been available for such analysis. Such a study was impeded by the confused state of the taxonomy of the group, until this was clarified by Smith (1939).

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University of Hawaii.

<sup>2</sup> Bertoni (1919) considered Paraguay as the country of origin of Ananas comosus. The view was expressed orally by a South American taxonomist who had specialized in the Bromeliaceae that Ananas and Pseudananas originated in the section of Paraguay, Argentina (Misiones), and Brazil (Paraná) called Guayra by the Guarani Indians.

The flora of South America is inadequately known, certain areas being unbotanized (Gleason, 1932), and this lack of information, evident for the Bromeliaceae as a family (Smith, 1934), is particularly conspicuous in the genera Ananas and Pseudananas. Perhaps because of technical difficulties in the collection and preservation of the fleshy flowers and fruits, these genera have been neglected entirely by many collectors, or specimens of the most fragmentary sort retained. All too many herbarium sheets consist of a single leaf and the general statement that the flowers were purple or lavender, thus giving no basis for evaluation.

This paper presents notes on the distribution and ecology of Ananas and Pseudananas with the hope of stimulating botanists to collect these plants in certain poorly known areas. These notes are based largely on field observations between October, 1938,

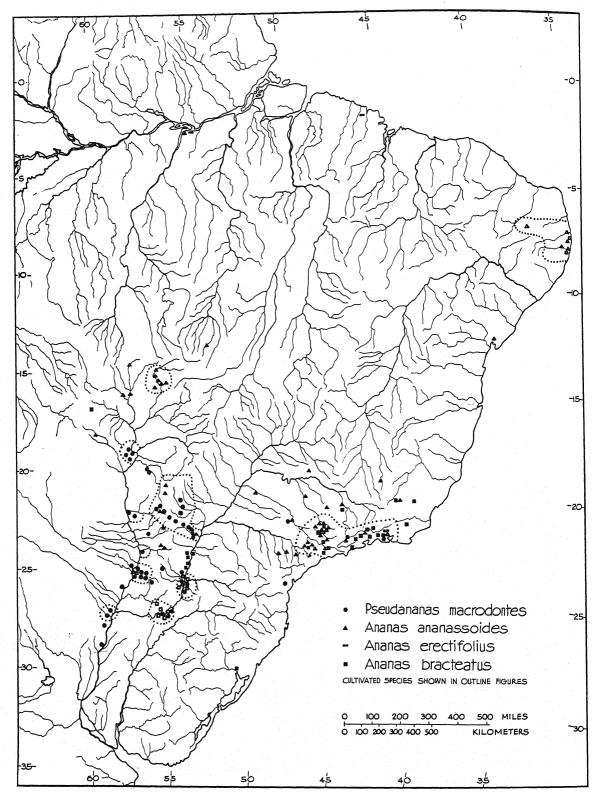


Fig. 1. Distribution of species of Ananas and Pseudananas in South America, based on field observations and on specimens in herbaria. Outline triangles in northeastern Brazil represent the ornamental A. ananassoides var. nanus. Outline squares on the Paraná River represent A. bracteatus var. typicus grown for fruit. Other outline figures indicate plants in botanical gardens and experiment stations. Areas within stippled lines were explored by the writers.

and March, 1939, during exploration for wild and cultivated forms of Ananas. Reliable data from specimens studied in the following herbaria have been incorporated: (1) Escola Superior de Agricultura e Veterinaria (Recife, Pernambuco, Brazil); (2) Field Museum of Natural History; (3) Gray Herbarium; (4) Instituto Biologico, Secção Botanico (São Paulo, Brazil); (5) Museu Argentino do Ciencias Naturales, Sección Botánico (Buenos Aires); (6) Museu de Historia Natural del Paraguay (Asuncion); (7) New York Botanical Garden; (8) United States National Arboretum (Department of Agriculture, Washington, D. C.); (9) United States National Herbarium. A few verified reports by competent observers have been included. While these data are not exhaustive, it is evident that extensive search among world herbaria would tend to increase reports from areas already covered rather than greatly to extend the distribution.

The nomenclature employed is that of Smith (1939), which was based in part upon the writers' herbarium material, photographs, and data. Dried specimens and photographs are filed in herbaria numbers 2, 3, 7, and 8 above as evidence of the species concepts followed. For brevity, the list of observed locations of the various species and of the verified recognizable herbarium materials is not presented, but is on file at the Pineapple Experiment Station, University of Hawaii, and at Gray Herba-

rium.

DISTRIBUTION.—The distribution of *Pseudananas macrodontes* (E. Morr.) Harms, *Ananas ananassoides* (Bak.) Smith, *A. bracteatus* (Lindl.) Schult., and *A. erectifolius* Smith is presented in figure 1. The varieties of the second and third species were not distinguished for this purpose. It will be noted that most of the locations fall between 14° and 29°S. latitude and between 59°W. and the east coast (39° to 50°W.).

The point of origin of three of the species probably lies within the area of 15° latitude and 9° to 20° longitude so delimited. More botanizing and field study will be required before the area can be more exactly located, but certain clues are afforded by this map. The proximity of P. macrodontes to A. ananassoides in southeastern Matto Grosso and to A. bracteatus in Misiones, Argentina, and southeastern Paraguay, and the contiguity of A. ananassoides and A. bracteatus in the Rio de Janeiro-São Paulo section suggest that all three species may be concomitant in the intervening territory.

P. macrodontes is centered largely in the drainages of the Paraná and Paraguay Rivers of Paraguay and adjacent Argentina and Brazil, but it extends eastward in a few places. Its spread to the west seems to have been limited by the salinity of the soil and streams and by the frequent droughts of the Chaco sections. The limiting condition in the east and northward along the coast is more obscure.

<sup>3</sup> Oral report by Teodoro Rojas and J. B. Jiménez, Museo de Historia Natural del Paraguay, Asuncion, who have botanized in the area.

In the interior the species is shut off on the north by the relatively dry, open Planalto of Matto Grosso and in the south by frosts, low temperature, and decreased rainfall.

A. ananassoides, the most generally widespread of the species, is apparently limited on the southwest by the decline of the Planalto on which it finds a favorable environment. An extension of this high zone southward into north central Paraguay accounts for the presence of A. ananassoides there. In certain areas along the margin of this plateau (e.g., the Campanario-Porto Felicidad section of Matto Grosso) A. ananassoides and P. macrodontes grow well and abundantly within a half mile of each other. Due to the abrupt transitions of rain forest and serrado<sup>4</sup> in southeastern Matto Grosso, this distribution is entirely compatible with the habitat of each.

From the known habitat of A. ananassoides, it seems probable that the species extends on the north until stopped by the Amazonian jungle. Information on the range in this direction and toward the northeast and southeast is very meager. A. ananassoides var. nanus was observed only as a cultivated ornamental in northeastern Brazil but was said to be wild in that region. If this be true, the range of the species is tremendously increased, but the point of convergence with P. macrodontes and A. bracteatus lies definitely further south.

A. bracteatus is known to occur in two distinct areas, one centering on the Alto Paraná and one in the Rio de Janeiro-São Paulo section of Brazil. In the former it occurs in proximity to P. macrodontes and in the latter to A. ananassoides. A. bracteatus probably will be found in the intervening territory, at present botanically almost unknown (Gleason,

1932).

A. bracteatus was almost always found in proximity to some habitation; on the Alto Paraná it occurred frequently in areas near former Jesuit missions. The obvious interpretation is that the plants have been spread and maintained by man. Antithetic to this, however, is the fact that the explored areas suitable for A. bracteatus are also favored agricultural regions and that the widespread practice of temporary utilization and abandonment of land (James, 1938) has decimated the species. The region between the Paraná River and the east coast has been less settled. It is, therefore, urgent that collections be made in the Brazilian states of Paraná and Santa Catharina before the present wave of colonization (Maack, 1938) has destroyed the native vegetation. One explanation for the present widespread survival of A. ananassoides is that it occurs on poor agricultural land; this tendency is commonly recognized and the plant utilized as an indicator of that condition by prospective purchasers. The lowland areas occupied by P. macrodontes also have been less intensively exploited.

<sup>4</sup> An ecological type characterized by tortuous trees (e.g., *Pithecolobium*) 10 to 15 feet high and brush in scattering open thickets; cacti are essentially absent and the sparse ground cover consists of grass and herbaceous plants. The soil generally is infertile and rainfall is low.



Fig. 2. Representative habitats of *Pseudananas* and three species of *Ananas* in South America. A (upper left). *Ananas comosus* wild or naturalized in open woods near Coxipo, Matto Grosso, Brazil. B (upper right). *Pseudananas macrodontes* under dense forest canopy at San Bernardino, Paraguay. C (lower left). *A. bracteatus* var. *typicus* growing at margin of clump of trees near Encarnacion, Paraguay. D (lower right). *A. ananassoides* var. *typicus* growing in the open brushy serrado near Mogy-Mirim, São Paulo, Brazil, showing inflorescences.

A. erectifolius is still too little known to warrant more than conjecture as to its distribution and affinities. It shows some similarities to the wild A. ananassoides and to the cultivated A. comosus which suggest that it may have evolved from those widely distributed species. More detailed information on the occurrence of these three forms in the southern

side of the Amazon drainage is needed to clarify this point.

A. comosus growing wild or semi-wild often has been reported from various parts of the tropics and sub-tropics. It should be noted that a great many of the important cultivated pineapple varieties will not survive such competition with other plants. This

species probably originated in or near the region discussed in this paper as the native home of the two genera. There is abundant evidence that the early peoples distributed the pineapple over a wide area, and Columbus is said to have introduced it into Europe from the West Indies. On this basis the occurrence of "wild" A. comosus may be dismissed as naturalized in many localities, as on the island of Hawaii and on Trinidad (Britton and Britton no. 2107) and probably also in British Guiana (Gleason no. 523) and Peru (MacBride no. 5482). Similar instances within or near the region regarded as the point of origin of the genus are more difficult to evaluate. The areas of A. comosus observed by the writers at Coxipo, Matto Grosso, and at Moura Brazil, Rio de Janeiro, are cases in point. The two distinct plants of these areas produced palatable fruit different from the naturalized pineapples observed at Prazeres and Tiuma, Pernambuco, and on the island of Trinidad. All of these sites were in or at the margin of semi-open stands of large trees, a shaded type of habitat similar to that of A. bracteatus. The plants were abundant, vigorous (fig. 2A), and occurred over large areas. It is uncertain whether these plants represent a link between the truly wild and the cultivated types, or merely strains of the latter retaining enough ancestral vigor to succeed untended. The widespread destruction of original ground cover, mentioned above, will make difficult the finding of intermediate forms.

The evidence presented above indicates that the key to more definite location of the point of origin of P. macrodontes, A. ananassoides, and A. bracteatus lies in the States of Paraná, Santa Catharina, and parts of São Paulo. The south side of the Amazon drainage is a second zone from which collections are needed for clarification of the distribution of

these genera.

Ecology.—The habitats of P. macrodontes, A. ananassoides, and A. bracteatus differ widely, a situation consistent with the variety of ecological conditions found in the probable area of origin designated above.

P. macrodontes is seen in interior lowland forests under canopies of variable density (fig. 2B). It occurs in plant associations ranging from the cactus-Erythrina-Prosopis type near Corrientes, Argentina, to dense rain forest near Campanario, Matto Grosso, Brazil. In the drier climate it was within a few yards to a half mile of the streams, dependent on the size of the body of water and the presence of a dense canopy and rather thick duff. Areas subject to overflow at high water were not occupied by P. macrodontes." whereas Bromelia and Aechmea were often observed in such locations. In areas of heavy rainfall the plants are not restricted to the streams or rivers and attain much larger size. The species, therefore, may be considered as adapted to humid lowland forests, but capable of withstanding considerable drought. In the Corrientes section, after three

<sup>5</sup> Bertoni (1919) reported a similar environment for this species.

phenomenally dry years, we observed numerous fertile fruits on stunted plants at a time when adjacent Opuntia was badly withered. Leaf mold was present almost everywhere this species was found; through this medium the plant sends stolons for distances up to six feet. This feature results in an open spacing within an area of P. macrodontes, as compared with the clumping evidenced by species of Ananas,7 and this spacing is distinctly advantageous in habitats

with reduced sunlight.

A. bracteatus is rather intermediate between Pseudananas and A. ananassoides in type of habitat. It does not grow in as profound shade as Pseudananas, being found on the fringes rather than extending far into a stand of trees8 (fig. 2C). There was some evidence of variability in capacity to withstand direct sunlight exhibited by representatives of this species from various sections of Brazil when growing at the Instituto Agronomico, Campinas, São Paulo. Although best development is attained in soil rich in leaf mold, the plant is often found in sites deficient in organic matter. The species tended to be restricted to depressions in which moisture obviously remained at a fairly high level; this condition was less noticeable in regions of abundant rainfall.

A. ananassoides represents the highest development of the genera in drought resistance. This plant is characteristically of the serrado of the Planalto. an area of tortuous stunted trees and brush in scattering open thickets (fig. 2D). Since the canopy over A. ananassoides is never dense, the plant is not handicapped by growth in large, dense mats. It also occurs in isolated clumps and occasionally was observed growing on termitaria in Matto Grosso. The latter habitat is an extreme example of the independence of the species in requirement of humus.

The habitat of A. erectifolius is conjectural, since the writers did not observe this species in the natural state. The fact that it is native in the Amazon area would suggest that it is a plant of the wet lowland. However, in gardens it made fairly vigorous growth in the drier region around Campinas, São Paulo.

The development of these species in such distinct natural habitats is an expression of their genetic differences. Unpublished records indicate that when

<sup>6</sup> The slender stolons bear chlorophyllous scales and progressively root at each node as the structure elongates. The terminal leafy rosette of the young plant is well formed before its basal roots appear, in striking contrast to the stolons of Bromelia which, under either aerial or subterranean conditions, form roots only at the base of the apical plantlet, and to the suckers or rootstocks of Ananas, which are relatively short and bear nodal roots. Aerial stolons are sometimes formed by Pseudananas (e.g., at San Bernardino, Paraguay) and in such cases act as those of *Bromelia*. The stolons of *Pseudananas* were found to decay earlier than those of Bromelia. These characteristic features supply an accurate field method of generic separation of non-fruiting plants, a task otherwise often extremely difficult.

<sup>7</sup> Of the most common bromeliaceous associates of these genera, Bromelia is spaced in the manner of Pseudananas,

and Aechmea is similar to Ananas.

A habitat similar to that reported by Bertoni (1919). <sup>9</sup> Corroborates the statement of Bertoni (1919).

A. bracteatus and A. ananassoides are crossed with a cultivated A. comosus, some of their progeny show adaptability to contrasting habitats unfavorable to many cultivated varieties, but resembling the natural environments of their respective wild parents.

#### SUMMARY

Evidence is presented that *Pseudananas* and most species of *Ananas* are native to that area of South America between 14° and 29°S. latitude and east of 59°W. longitude.

From exploration and study of herbarium material the distribution of species of *Pseudananas* and *Ananas* has been determined. *P. macrodontes* was found principally in the moist lowland forests of the Paraná-Paraguay Basin. *A. ananassoides* occurred in the dry, poor soils of the serrado of the Brazilian Planalto. *A. bracteatus* was observed in the better agricultural areas of the Rio de Janeiro-São Paulo

section and on the Alto Paraná, growing in moist soil at the margins of tree clumps; data on its natural distribution is limited because of prevalent agricultural practices and inadequate botanization. The habitat of A. erectifolius is probably the lowland rain forest of the Amazon drainage.

P. macrodontes was contiguous with A. ananassoides in southeastern Matto Grosso and with A. bracteatus in northeastern Argentina and southeastern Paraguay. The latter two species occurred together in the Rio de Janeiro-São Paulo region of Brazil. A central area common to all may be found with adequate botanization of the Brazilian states of Paraná, Santa Catharina, and parts of São Paulo. Clarification of the distribution of A. erectifolius awaits exploration of the southern side of the Amazon drainage.

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# EXPERIMENTS ON BUD INHIBITION WITH INDOLE-3-ACETIC ACID <sup>1</sup>

## Folke Skoog

RESULTS OBTAINED by several workers with numerous species have established that correlative inhibition, as for example, the inhibition of the development of lateral buds by the terminal shoot in Vicia and Pisum, is due to auxin produced in relatively high concentrations by the inhibiting organ (Thimann and Skoog, 1933, 1934; Müller, 1935; Dostal, 1936). However, no agreement has been reached as to the action of auxin in inhibition. Ten different mechanisms have been proposed (ref. Thimann, 1939). These fall mainly into two groups according to whether auxin is considered to act directly in the inhibited organ (concentration effect, Thimann, 1937) or indirectly through affecting the availability or the translocation of nutrients to the inhibited organ (Laibach, 1933; van Overbeek, 1938) or the mobilization of more specific growth factors (Went, 1936, 1939; Ferman, 1938). An alternative indirect mechanism postulating that an inhibiting substance is formed under the influence of auxin has been proposed by Snow (1937).

Since the evidence is not conclusive for any of the above mechanisms, the question of whether the ac
1 Received for publication July 19, 1939.

tion of auxin in inhibition is direct or indirect has been further investigated.

MATERIALS AND METHODS.—Embryos of Pisum, variety Alaska, were prepared and grown under sterile conditions. The seeds were immersed in 0.1 per cent mercuric chloride solution for 20 to 30 minutes, rinsed, and allowed to soak in water for about 12 hours. They were then dipped in 90 per cent alcohol and rinsed in water immediately before removal of the embryos. The buds were cut with a sharp knife in the hypocotyls well above the cotyledonary axils, so that no root or cotyledonary tissues adhered. They were grown usually in lots of 3 in 125 cc. Erlenmeyer flasks containing 40 cc. of White's (1938) nutrient solution.2 Indole-3-acetic acid was supplied separately to prevent a possibility of its inactivation by heat. It was prepared by dissolving a given weight of crystals in peroxide free ether, evaporating to dryness in a sterile flask, and taking up in a given volume of water. A series of dilutions were made, and 1.0 cc. of the desired con-

 $^2$  Prepared according to White's suggestion with slight modifications in salt concentrations, with only one amino acid (glycine,  $^2$  mg./l.) and with 0.1 mg./l. vitamin  $B_1$ .

centration was added to each culture flask. To the controls was added 1.0 cc. of water. Since the preparation of the cultures required several hours, buds removed early and late were distributed equally among the different sets of an experiment. The initial lengths of the buds after transference to the solutions varied from 2.0 to 3.5 mm., but more than 90 per cent were between 2.5 to 3.0 mm. long. The buds were grown in a high humidity dark room at 25°C. and were exposed to light only at intervals for measurement of their lengths. Measurements given below are based on means of from 12 to 16 plants, which numbers have been found satisfactory for the periods of growth to be considered. An estimate of the reproducibility of results may be gained from some duplicate sets which are included.

RESULTS.—The buds grew for periods of at least four weeks with a constant or increasing growth rate and in no case with a decreasing average growth rate, although individual plants might show a decrease, and the rates were always less than would have been obtained in the presence of seeds. Whatever factors are needed for continued growth are therefore either supplied by the nutrient solution or are present in the buds for the duration of the experiments. Consequently, this material offers an opportunity to determine effects of auxin on growth

exerted directly in the buds.

The effect of different concentrations of indole-3acetic acid on the growth in length of the buds is indicated by results of an experiment in figure 1. In the first few days usually all treated plants grew less than the controls. Thereafter, those with the lower concentrations grew as fast or somewhat faster than the controls, whereas the plants with higher concentrations remained strongly inhibited for longer periods. For the purpose of demonstrating an inhibiting effect of applied indoleacetic acid the cultures need be grown for only a few days, and this early inhibition is considered below. However, in order to observe the effect of the treatments on subsequent growth, the cultures were kept for longer times. With concentrations below about 0.1 mg./l. of indoleacetic acid, the lengths and fresh weights of the treated plants would approach the values for the controls within four to six weeks, but with higher concentrations present continuously the final measurements remained less than those of the controls. If, on the other hand, plants were supplied with the higher concentrations, 10.0 and 0.1 mg./l., respectively, for shorter intervals, no permanent decrease in growth occurred. Results of one such experiment are presented in table 1. It may be seen that after exposure to even the 10.0 mg./l. solutions for as long as 65 hours, the buds subsequently grew as much as the controls, and after exposure to this concentration for a week, they still showed an increased growth rate upon removal but did not reach the final lengths or fresh weights of the controls. Thus the inhibiting effect of indoleacetic acid obtained during the first few days after application is reversible over a wide range of concentrations and is not due to injury or toxicity.

The above data further indicate that the application of very dilute solutions of indoleacetic acid continuously or of stronger solutions for short intervals resulted in a stimulation of bud growth. This effect was present in four out of six experiments but appeared in the late stages when the differences in

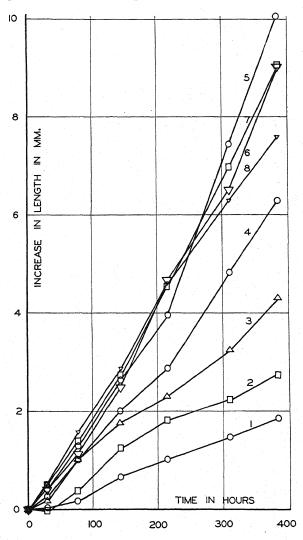


Fig. 1. Effect of concentration of indole-3-acetic acid on the growth of excised buds of Pisum. Curve no. 1, 10 mg./l.; no. 2, 2 mg./l.; no. 3, 0.4 mg./l.; no. 4, 0.08 mg./l.; no. 5, 0.016 mg./l.; no. 6, 0.0032 mg./l.; no. 7, 0.0006 mg./l.; no. 8, 0.0000.

length of the individual plants were large, so that a statistically significant increase was obtained only in one case. An additional experiment with a few treatments of from 40 to 60 plants each also failed to show a real increase within 25 days. If the stimulating effect observed is real, its presence must require some condition as yet not adequately controlled. In any case, the increase was never so large as, and is probably not comparable with, the stimulation of bud growth obtained by Went (1939) by the ap-

Table 1. Effect of concentration and time of treatment with indole-3-acetic acid on growth of excised Pisum buds.

				Ho	urs o	f mea	surements				
		42	92	136	213	280	381		670		
Conc.	Hours in								Mean fresh	No. c	of roots
IAA. mg./l.	IAA. solutions	Mean increase in length of buds in mm.					wgts. (mg.)	Mean	Largest no.		
	6	0.64	1.42	2.7	5.0	6.5	$8.8 \pm 0.6$	18.0	52	1.6	3
0.00	65	0.66	1.89	2.9	5.9	7.3	$9.7 \pm 1.1$	22.7	61	1.6	2
	Continuous	1.02	1.91	2.7	5.5	6.7	$10.1 \pm 0.5$	27.9	53	1.7	2
	( 6	1.08	2.19	3.3	6.0	9.5	$12.6 \pm 1.1$	27.6	62	1.7	2
	16	1.00	2.20	3.7	6.5	9.9	$13.2 \pm 0.8$	37.3	72	1.4	2
	28	0.49	1.65	2.9	5.7	8.0	$10.0 \pm 0.6$	24.6	50	1.4	2
0.10	65	0.28	1.73	2.8	5.3	7.4	$9.5 \pm 0.9$	23.8	53	1.0	2
	165	0.42	1.13	1.7	4.2	6.6	$9.2 \pm 0.9$	20.2	49	1.4	. 3
		(0.42	1.19	2.0	3.2	5.0	$7.2 \pm 0.6$	18.6	58	0.6	1
	Continuous	0.38	0.89	2.0	2.8	4.0	$7.1 \pm 0.5$	20.6	57	1.7	3
	6	0.70	1.59	2.6	4.5	7.8	$11.6 \pm 1.5$	30.7	72	2.0	3
	16	0.58	1.53	2.4	4.0	6.9	$9.5 \pm 0.8$	16.2	47	2.8	6
	28	0.29	1.48	2.3	4.2	6.7	$9.9 \pm 0.4$	15.0	52	2.3	5
10.0	65	0.15	1.34	2.3	4.8	7.8	$10.9 \pm 1.0$	16.8	56	2.3	4
	165	0.33	0.60	0.9	1.9	3.7	$4.9 \pm 0.6$	7.5	26	1.7	4
	0	(0.11	0.46	0.9	1.3	2.0	$2.4 \pm 0.2$	2.7	12	0.1	1
	Continuous	0.21	0.78	1.2	1.5	2.0	$2.4 \pm 0.4$	3.2	12	0.0	0

plication of weak auxins to the stems of decapitated Pisum plants.

The present experiments show, nevertheless, that a reversible inhibition of bud growth is produced consistently by the proper concentrations of indole-acetic acid under conditions where the effect cannot be due to a diversion of nutrients or growth factors from one organ to another but must be exerted in the inhibited organ itself.

A brief reference to root development is made in this connection. No roots were present on any plants during the first week in which the marked inhibition of bud growth, considered here, was obtained. Most plants developed one or more adventitious roots within the second or third week. Usually, as indicated in table 2, the intermediate concentrations of indoleacetic acid led to a definite increase in the number of roots formed and a decrease in root elongation. The lower concentrations tended to hasten, the higher to delay, the time of appearance of roots. In cultures remaining after six weeks, however, some

roots had been formed in all but the 10 mg./l. concentration. The appearance of roots may contribute to a subsequent acceleration of growth of the stems. Data presented in table 3, obtained from 16-day-old cultures, show that plants with roots are in general longer than those without roots, but also some of the latter are longer than the average. Thus, inhibition of bud growth by indoleacetic acid is obtained in the absence of roots. The roots do not appear and are not essential for continued growth in length of these buds for a period much longer than required to demonstrate a marked inhibiting effect. It is concluded that roots can be disregarded entirely in a consideration of the inhibiting action of indoleacetic acid in the present experiments.

The inhibition of buds obtained here is directly comparable with that obtained by the application of auxin in lanoline paste directly to lateral buds of decapitated *Pisum* plants (Thimann, 1937). It is due principally to a reversible inhibition of growth of the entire organ rather than to a redistribution of

Table 2. Effect of indole-3-acetic acid on root growth on excised Pisum buds.

Age of cultures		Con	centrat	ion of indo	le-3-acet	ic acid ir	n mg./l.		
in hours	10.0	2.0	0.40	0.08	0.016	0.003	0.0006	0.0000	
			P	verage ni	ımber of	roots pe	r plant		
311	0	0	0	0	0.7	0.8	0.3	0.4	0.1
384	0	0	0	0.4	0.9	1.5	0.6	0.5	0.3
520	0	0	0	1.1	1.7	1.6	0.6	0.5	0.3
				Average	lengths o	f roots in	mm.		
311	0	0	0	0	4.7	6.1	7.0	23.3	11.5
384	0	0	0	2.6	7.0	9.3	12.3	22.4	14.8

growth among its component tissues, although within a range of the weakly inhibiting concentrations a considerable growth in thickness may occur. The inhibition in the present experiments was never complete even with the higher concentrations, but this might be due merely to the fact that the buds

Table 3. Relation between growth in length of buds and presence of roots. (Age of cultures 16 days.)

Conc.	a with,	Bud	NT		
IAA. mg./l.	b with- out roots	Longest	Shortest	Mean	No. of plants
0.00	{a }b	20. 14.	13. 7.	17.2 10.6	4 12
0.00	{a }b	18. 15.	12. 9.	15.7 11.0	3 5
0.0006	{a }b	25. 13.5	9.5 9.	15.7 11.0	8
0.003	{a }b	19. 15.	7. 12.	13.5 13.5	13 3
0.016	{a }b	22. 20.	9. 10.	14.9 14.5	10 6
0.08	{a }b	12. 20.	11. 8.5	11.5 10.2	4 13
0.4	b	9.0	5.5	7.3	16
2.0	b	8.0	3.5	5.8	16
10.0	b	5.5	4.0	4.8	16

had started to grow before the indoleacetic acid was added so that its effect would be less; this is true also for lateral buds (Thimann and Skoog, 1934; van Overbeek, 1938). What growth did occur was limited to the lowest internode, which, nevertheless, remained much shorter than in the controls. There is, therefore, no evidence that the function of applied auxin in the inhibition of excised buds differs in any essential respect from that in the inhibition of buds on decapitated plants, although important secondary differences may exist. On the contrary, the following evidence indicates that the function is the same in the two cases.

Auxin has been demonstrated to cause inhibition of lateral bud development only when applied to stems in relatively high concentrations and only under conditions where it may reach the buds. Since the latter point has an important bearing on various proposed mechanisms of bud inhibition, some data will be presented regarding the effectiveness of indoleacetic acid applied to various positions of the stem. As shown by the results of two experiments (table 4), which have been repeatedly confirmed, concentrations of 2 or 4 mg. per gram of lanoline paste applied to stems of decapitated Pisum, grown in the dark, were completely effective in inhibiting the development of lateral buds for seven days when applied to the cut surfaces and also about 70 per cent effective when applied immediately below the cut surfaces; on the other hand, they were without any inhibiting effect when applied to the stems below the lateral buds. In other experiments, plants were treated with plain lanoline or auxin pastes below the lateral buds, and later the developing buds were decapitated or removed. In the control plants cotyledonary buds then developed, but in the treated plants only secondary lateral buds grew out. The treatment, therefore, was effective but only below the point of application. When, on the other hand, the basal treatments were with aqueous solutions, from which upward movement of the indoleacetic acid is facilitated through transport in the xylem, inhibition of the lateral buds above the point of application was obtained. The failure of the basal applications in lanoline paste must therefore be ascribed to insufficient upward movement of the substance to reach the buds, due to the existing polarity of transport in the stem. The same conclusion may be drawn from observations on the development of lateral buds in ringed plants.

Snow (1937), however, has shown that in a stem split lengthwise, so that auxin produced by a growing shoot must move acropetally to reach the lateral bud, the latter was nevertheless inhibited. On the basis of this and his earlier findings indicating that the extent of inhibition of lateral buds increases with distance from the apex, he has proposed that

Table 4. Relative effectiveness of indole-3-acetic acid applied to different positions on the stem of Pisum in inhibiting lateral bud development.

				Position of	application of	lanoline pas	te to stems		
		To cut su	rfaces	5 mm. belo	w cut surface	s 5 mm. belov	v upper bud	5 mm. below	lower bud
Exp.	Conc. IAA.	The state of the s		Le	ngths of later	al buds in mn	n.	***************************************	
No.	in mg./g.	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper
ıı ıı	2 4	1.7±0.6 1.0±0.0	2.5±0.9 2.6±0.3	1.6±0.1 1.2±0.1	$22.7 \pm 5.2$ $23.1 \pm 5.1$	1.9±0.2 2.1±0.3	51.2±5.0 47.4±4.7	1.6±0.1 7.6±0.4	65.1±5.4 83.2±8.2
I	0	******	•••••	$1.7\pm0.2$ $5.1\pm2.1$	$73.9 \pm 6.4$ $78.8 \pm 9.0$			$2.3\pm0.3$ $1.5\pm0.2$	$65.2 \pm 7.4$ $65.5 \pm 4.7$
II	0 0			of treatmen below buds	ts} Lower	$\begin{cases} 1.0 \pm 0.1 \\ 1.0 \pm 0.0 \end{cases}$	Upper	$ \begin{cases} 1.0 \pm 0.1 \\ 1.8 \pm 0.3 \end{cases} $	

Measurements of Experiment I, 6 days, Experiment II, 7 days after application. Each figure is a mean of from 25 to 30 plants. The deviations represent standard error of the mean  $= \pm \left[\sum d^2/n(n-1)\right]^{1/2}$ .

Table 5. Relation between stem swellings and growth of lateral buds in decapitated Pisum.

Conc. IAA. mg./gr.	No. of plants with bud lengths	Increase in length of buds in mm.	Relative vols. of stem swellings $(1  imes  ext{d}^2)$
		(mean)	(mean)
10.0	Above average 1 Below average 9	0.0 0.7	\{309\\276\}
2.5	Above average 6 Below average 12	$\begin{cases} 15.4 \\ 0.0 \end{cases}$ 5.1	${227 \brace 168}$ 188
0.63	Above average 8 Below average 9	$ \begin{array}{c} 20.1 \\ 1.6 \end{array} $	$   \left\{     \begin{array}{c}       138 \\       90   \end{array}   \right\} $ 113
0.16	Above average 9 Below average 10	42.3 $4.9$ $23.7$	$ \left\{\begin{array}{c} 86\\59 \end{array}\right\} $
0.04	Above average 12 Below average 10	109.7 $33.1$ $74.9$	$ \left\{\begin{array}{c} 69\\63 \right\} $
0.01	Above average 13 Below average 10	125.8) 28.5	$\left\{\begin{array}{c} 16\\13 \end{array}\right\} $ 15
0.00	{Above average 5 Below average 5	164.4 $120.0$ $142.2$	$\left\{ egin{array}{c} 4 \\ 4 \end{array} \right\}$

the action of auxin is not direct but is due to the formation in its presence of an inhibiting substance which moves unrestrictedly through the plant causing inhibition where it is present in relatively large proportion to the auxin. The distance effect has been supported by Went's experiments in which weakly inhibiting concentrations of auxins were applied to cut surfaces of Pisum stems decapitated at different distances from the buds. But he interprets the effect as due to the attraction of growth factors away from the buds to the points of auxin application. Went further showed that slightly higher concentrations were effective whether applied to long or to short stems, and he confirmed the earlier observation (Thimann, 1935) that auxins which are less readily transported through the plant are effective only when applied near to the buds. The latter findings, as well as the data in table 4, are not in agreement with the theory of Snow in its present form,3 nor do they support the diversion theory of Went.

Results of a different nature presented in part in table 5 are also in disagreement with these mechanisms. A series of concentrations of indoleacetic acid in lanoline paste were applied to cut surfaces of decapitated Pisum stems in the dark, under which conditions apical swellings of the stems resulted from the treatments. However, in the upper range of concentrations where the extent of bud growth was affected relatively little, there were still large increases in the size of stem swellings, so that apparently bud inhibition may be very marked when there is only a partial mobilization of growth factors toward the apex. It was found, further, that when the plants of each concentration were considered separately, the plants with the biggest swellings had also the longest buds-i.e., a positive correlation exists between size of swellings and length of buds. On the

3 An inhibitor which is evidently an auxin complex has

recently been reported (private communication, Calif.

Inst. of Technology).

basis of diversion theories postulating that inhibition of buds results from a depletion of food factors in the auxin induced growth of the stem, a negative correlation would be expected. On the basis of Went's diversion theory a positive correlation can be expected only on the assumption that entirely separate limiting factors are involved in the production of swellings than are required for bud growth, and further, that in a given plant the presence of large amounts of the former factors is associated with relatively large amounts of the latter.

Finally, both the above theories fail to account for the very general phenomenon of bud inhibition in geotropically stimulated stems, where bud development is confined mainly to the upper sides even though auxin is derived from both sides and is present in higher concentrations on the lower sides of the stems.

In these diverse cases a direct action of auxin in the inhibited organs offers a plausible explanation, except for the inhibition of one developing shoot by another. In this case the latter mechanism appears insufficient but may not be so in fact if the inhibition is not merely a concentration effect. The experiments of van Overbeek (1938) and Ferman (1938) strongly indicate that in correlative inhibition the function of auxin cannot be ascribed to its concentration alone, but that some additional factor must be included in formulating a mechanism of general application. The present experiments, however, show that indoleacetic acid can inhibit the growth of excised buds, and they permit the deduction that this action of auxin directly in buds is probably of general occurrence, whether or not the effectiveness of auxin in correlative inhibition is limited to concentrations higher than required for growth, whether it is influenced by an effect on translocation, or whether it is determined by other factors.

#### SUMMARY

Excised buds of *Pisum* cultured in White's nutrient solution continue to grow in the dark for periods of several weeks. They therefore offer an opportunity to determine effects of auxins exerted directly on buds.

Indole-3-acetic acid was added to the nutrient solutions in concentrations from 0.0006 to 10.0 mg./l. All but the lowest concentrations inhibited the growth of the buds very markedly during the first few days after application. The inhibition obtained during this time was reversible and was not due to toxicity.

The weak and also the stronger solutions perhaps caused a subsequent stimulation of growth if the buds were transferred from the latter to plain nutrient solutions after short periods, but if the buds were kept in solutions higher than 0.1 mg./l., they

remained shorter than the controls for the duration of the experiments.

Further experiments with *Pisum* seedlings and other evidence are presented which indicate that auxin applied to stems also must reach the lateral buds to inhibit their growth.

It is concluded that even though the inhibiting action be not explainable in terms of concentrations alone, indoleacetic acid can inhibit growth directly in buds; and that whether or not the effectiveness of auxin in correlative inhibition is influenced by an effect on translocation of nutrients or by other factors, auxins applied to decapitated stems probably generally exert an inhibiting action in the lateral

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## SEX CHROMOSOMES OF CANNABIS SATIVA 1

## Elizabeth L. Mackay

Cannabis sativa was reported by Strasburger (1909, 1910) to have no sex chromosomes. He showed the haploid number to be 10, the somatic number 20. McPhee (1924) likewise found no sex chromosomes. Working with two Japanese varieties of hemp, Hirata (1924, 1929) observed an unequal pair of chromosomes in the metaphases of the heterotypic division in pollen mother cells. Since no unequal pair was found in the female, but instead an equal pair slightly larger than the other chromosomes, he assumed this large pair in the female to be an XX and the unequal pair in the male XY. The presence of an unequal pair in Cannabis was con-

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firmed by Sinotô (1928, 1929). Driga (cited by Hoffmann, 1938), investigating a large number of races, found in the male an unpaired three-parted chromosome together with a two-parted chromosome.

Sex chromosomes have not been clearly distinguished in somatic cells. Breslawetz (1932) saw, in somatic divisions in the root tip, one large chromosome which she named the X and another with a median constriction which was smaller than the X but larger than the other chromosomes; this she identified as the Y.

In view of the discrepancy between the observations of Strasburger and McPhee on the one hand, and those of Hirata and Sinotô on the other, it has been suggested by various writers (e.g., Hoffmann, 1938) that varieties of hemp may differ in that some possess and some lack sex chromosomes. Because of

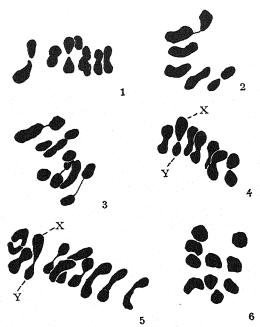


Fig. 1-6. Cannabis sativa.—Fig. 1-5. Lateral views of meta-anaphases of the first meiotic division of pollen mother cells. XY pair of unequal size.—Fig. 5. Aceto-carmine preparation showing unequal pair.—Fig. 6. Polar view of metaphase showning n=10.—Fig. 1-4, 6,  $\times$ 3650. Fig. 5,  $\times$ 3000.

this uncertainty, and particularly since no report has appeared of the occurrence of an XY pair in

American races, it seemed worth while to undertake the study reported in the present paper.

Material of staminate buds was fixed from two different wild stands of hemp in or near Madison, Wisconsin, and from one cultivated variety which was obtained from Professor A. H. Wright of the Agronomy Department of the University of Wisconsin. In favorable lateral views of the metaphases and anaphases of the first meiotic division in pollen mother cells of plants from each location, an unequal pair of chromosomes was very evident (fig. 1-5). Ten pairs of chromosomes are present, as described by the earlier workers already cited. Nine pairs are similar in size, each being composed of equal members. The tenth pair (XY) consists of one chromosome of about the same size as the members of the other nine pairs, and one much larger chromosome.

It is evident that this unequal pair is the sex chromosome pair. From the number of varieties from various parts of the world now known to possess an XY pair it is highly probable that this condition prevails in all varieties of *Cannabis sativa*.

#### SUMMARY

An unequal (XY) pair of sex chromosomes is present in staminate plants of probably all varieties of Cannabis sativa.

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# THE RELATION OF pH AND TEMPERATURE TO THE FRUITING OF PHYSARUM POLYCEPHALUM<sup>1</sup>

William D. Gray

THE FACTORS initiating and influencing the formation of fruiting bodies of myxomycetes have been considered by several workers, and various reasons have been advocated as being responsible for this process. Harper (1914) has shown that plasmodia of Didymium melanospermum (Pers.) Macbr. lose water when about to fruit, and for this reason Skupienski (1920) stated that sporangial formation is the result of desiccation and that small plasmodia will fruit more quickly than large plasmodia because they may lose water more easily. It is rather doubtful that such is the case, because Harper specifically pointed out that this desiccation is not simply the drying out of a colloidal mass but is probably due to the forcible extrusion of water from the protoplasm; this extrusion of water he thinks may be brought about by condensation processes involving changes from highly hydrated proteins to lipoid or other fatty storage products. The work of Constantineanu (1906) would also tend to discredit Skupienski's view, since this worker showed that plasmodia of Physarum didermoides (Ach.) Rost. and Didymium squamulosum (Alb. & Schw.) Fr. (D. effusum Link) would form fruiting bodies if the substratum was moist but would encyst if it was dry. Camp (1937) has shown that plasmodia of Physarum polycephalum Schw. may be induced to fruit if they are starved. This is easily demonstrated for this species and may be the factor largely responsible for some fruitings in nature; however, since most naturally occurring fruitings (and this is particularly true for forms that grow on wood) are to be found closely associated with easily available food material, we must look to other factors for the initiation of the fruiting process. In an earlier paper by the writer (Grav, 1938) it was pointed out that in general, light is necessary for fruiting of several yellow-pigmented plasmodial types. It is the purpose of the present investigation to determine the influence of the factors of pH and temperature upon fruiting. Other factors are undoubtedly also involved, since during its plasmodial stage a myxomycete consists simply of a mass of protoplasm, unprotected by any type of cell wall, and would naturally be expected to be extremely sensitive to numerous environmental factors.

MATERIALS AND METHODS .- Because of the ease with which it may be cultured, Physarum polycephalum Schw. was used as the experimental organism. Starved cultures were used in all experiments, since

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fed cultures are far too cumbersome to permit the handling of many in a single series. The difficulties to be encountered in the handling of numerous fed cultures simultaneously are obvious in view of the fact that one plasmodium may well fill a ten-inch culture dish within a few days. Starved cultures were prepared in moist chambers consisting of inverted embryological staining dishes (4  $\times$  4  $\times$  1.5 cm.), covered with porous towel paper, and placed in 500 cc. lipless beakers, to each of which 30 cc. of distilled water or buffer solution had been added. After small bits of plasmodium had been placed in each of the moist chambers, cellophane was placed over the top of each beaker and fastened by means of rubber bands. All cultures used in the following experiments were inoculated from five or six day old stock plasmodia which had been maintained in complete absence of light. Stock cultures were maintained in large culture dishes in the manner suggested by Camp (1936); such cultures were fed by sprinkling with rolled oats and presumably received an abundance of all the elements essential for their growth. Inoculations were made approximately twenty-four hours after the last feeding of stock plasmodia.

For the maintenance of various pH values in the moist chambers, the dibasic sodium phosphate-citric acid buffer mixtures recommended by McIlvaine (1921) were used; these were diluted 1:10. Such buffer solutions were always checked with the Beckman glass electrode. Values ranging from pH 3.0 to pH 8.0 (at intervals of one pH unit) were used.

Constant temperature was obtained through the use of a large tank of water, beakers being immersed in the water to a depth of about 5 cm. This tank was equipped with heating bulb, thermal regulator, stirrer, and a large copper coil through which tap water could be circulated. Cultures placed in the water bath were continuously exposed to light from a 300 watt incandescent bulb suspended one meter above the tank. Experiments were conducted at 21.0°, 25.0°, 27.5°, 30.0°, 32.5°, and 35.0°C. in the water bath; for checks which were run in complete absence of light, a small electric oven was employed. In addition to cultures maintained at the temperatures mentioned above, several hundred cultures were placed in the dark in a refrigeration room where the temperature generally remained at 8.0°C., although it sometimes rose as high as 12.0°C.

In the data presented in this paper, it will be noted that all figures expressing hours required for fruiting or for killing are averages taken from a number of cultures. In order to express the variation within each series, all data were analyzed by standard statistical methods, and with each average the probable error of the average is also listed.

INFLUENCE OF PH UPON FRUITING.—Preliminary experiments using cultures maintained at pH values ranging from pH 3.0 to pH 8.0 were run at 25.0°C., and it was found that a higher percentage of cultures fruited at the lower pH values (3-5) and also that less time was required for fruiting. Fruiting bodies appeared in cultures maintained at pH values ranging from pH 3.0 to pH 7.0, but in no case was fruiting obtained at pH 8.0. Few observations have been made concerning the effect of pH on myxomycetes, but the above findings are in general agreement with the findings of other workers. Howard (1931), when first describing methods for the culture of P. polycephalum, stated that plasmodia seem to prefer a slightly acid medium and will tolerate an acidity on oat agar as great as pH 4.8; the present work reveals that this species will tolerate a much greater acidity when starved cultures are used. Jahn (1932), referring to a species of Badhamia, which he cultured on agar with a decoction of Polyporus versicolor, observed that plasmodia showed injury when the pH of the medium was below pH 4.0 or above pH 7.5. Perhaps the most extensive observations on the effect of pH on fruiting were made by Emoto (1938), who collected about 4,000 different fruitings (consisting of 106 different species and varieties of myxomycetes from decaying wood representing 76 species of woody plants) and found that in most cases the reaction of the substratum was pH 4.2-5.8. Of these 4,000 fruitings, only 10 were found on substrates where the reaction was higher than pH 7.0. From the work of Smart (1937) it is apparent that an acid medium is also more favorable for spore germination; this worker conducted studies on spore germination with 70 species and varieties of myxomycetes and found that the optimum in all cases was between pH 4.5 and 7.0.

INFLUENCE OF TEMPERATURE UPON FRUITING .-In regard to the effect of temperature upon fruiting few observations have been made. It is to be expected that different species might have different optimum temperatures, and the reports of various workers would tend to bear this out. For example, Howard (1931) stated that the optimum temperature for P. polycephalum is between 20.0° and 26.0°C., whereas Skupienski (1934) found that the optimum temperature for Didymium xanthopus (Ditm.) Fr. is 10°-12°C. Constantineanu (1906) reported fruiting by P. didermoides at 8°-12°C. and in Fuligo septica (L.) Weber [Aethalium septicum (L.) Fr.] at 25° and 35° but did not state the optimum temperature for fruiting of these species. Skupienski also pointed out that physiologic strains may exist and showed that while the optimum temperature for D. xanthopus is 10°-12°C., a strain of the same species which he terms var. thermophilum (not used in a systematic sense) has its optimum at 25°C.

INTERRELATIONSHIP OF PH AND TEMPERATURE IN RESPECT TO FRUITING.—Shortly after this work was begun it became apparent that in their effects upon fruiting the factors of pH and temperature are

rather closely related and either may be considered a limiting factor with respect to the other. Results of experiments in which this interrelationship is considered are presented as follows:

Series I. Temp. =  $21.0^{\circ}C$ . (110 cultures).— Since Howard stated that the optimum temperature for P. polycephalum is between 20.0° and 26.0°C... the first of the present series was conducted within this temperature range. As stated before, pH values at this temperature, and all subsequent ones, ranged from 3.0 to 8.0 at intervals of one pH unit. At the first four pH values fruiting bodies were formed in all cultures, at pH 7.0 fruiting occurred in 60 per cent of the cultures, and at pH 8.0 sporangia appeared in only 55 per cent of the cultures. The average time required for fruiting (time being measured from the time of inoculation to maturing of fruiting bodies) was 30.1 hours at pH 3.0, dropping to 26.4 hours at pH 4.0, and gradually rising to 33.1 hours at pH 8.0.

Series II. Temp. =  $25.0^{\circ}$ C. (105 cultures).—No fruiting was obtained at pH 8.0 when cultures were maintained at this temperature. As in the preceding series, 100 per cent fruiting occurred in cultures conducted at pH 3.0 and pH 4.0; percentage fruiting then decreased from 93.3 per cent at pH 5.0 to 13.3 per cent at pH 7.0. At each pH value the average time required for fruiting was longer at this temperature than at 21.0°C.; for example, at 21.0° the average time for fruiting in cultures at pH 3.0 was 30.1 hours, while at 25.0°, at the same pH, the time required was 30.3 hours, a very slight difference to be sure, but probably quite valid since much greater differences were exhibited at higher pH values. As in Series I, fruiting at pH 4.0 required the least amount of time.

Series III. Temp. = 27.5°C. (146 cultures).—Cultures were run at the same pH values as in the preceding series, but fruiting was obtained only at pH 3.0, 4.0, 5.0, and 6.0. At pH 3.0 sporangia appeared in all the cultures, the percentage of cultures fruiting then decreasing with rise in pH value to only 77.3 per cent at pH 6.0. Cultures at pH 4.0 required the least time for fruiting, and at each pH value a greater time was required for this process than was required by similar cultures in the preceding series. The differences in time required for fruiting were in general greater between this series and Series II than between Series I and Series II; these differences are shown in table 1.

Skupienski (1930) has shown that variations in temperature produce profound changes in the structure of fruiting bodies of  $Didymium\ nigripes$  (Link) Fr., and it is interesting to note that similar morphological changes undoubtedly occur in sporangia of P. polycephalum. Although detailed observations of the structure of sporangia obtained in these experiments were not made, it should be noted that in this series ( $T=27.5^{\circ}$ ) and in all series where higher temperatures were involved, many sporangia were either extremely short-stipitate or sessile. More careful observations on this point will be made later.

Table 1. Differences in hours required for fruiting between Series I and Series II and between Series II and Series III.

pH	3.0	4.0	5.0	6.0	7.0	8.0
Series I, T = 21°	30.1	26.4	26.5	29.3	30.7	33.1
Series II, T = 25°	30.3	29.2	31.1	34.9	49.0	
Difference	0.2	2.8	4.6	5.6	8.3	٠
Series II, $T = 25^{\circ}$	30.3	29.2	31.1	34.9	49.0	٠.٠.
Series III, T=27.5°.	36.1	32.6	35.8	45.5		
Difference	5.8	3.4	4.7	10.6		

Series IV. Temp. = 30.0°C. (175 cultures). No mature fruiting bodies were obtained at this temperature at pH values higher than 5.0, although in some cultures at pH 6.0 sporangia were formed; these sporangia, however, never matured and are referred to in table 2 and elsewhere in the paper as "attempted fruiting." Percentage fruiting at pH 3.0 was 87.8 per cent as compared with 80.0 per cent at pH 4.0 and 47.2 per cent at pH 5.0. As in all the preceding series, cultures at pH 4.0 fruited in less time than cultures at pH 3.0 and pH 5.0 but required a longer period of time than similar cultures run at 27.5°. Table 2 shows the relationship of pH and temperature to fruiting in so far as percentage of cultures forming sporangia is concerned, while table 3 shows this same relationship with respect to time required for fruiting. Figure 1 illustrates the curves of fruiting derived from the data in table 3.

Series V. Temp. = 32.5°C. (207 cultures).— At this temperature sporangia were formed only in cultures maintained at pH 3.0 and 4.0, although

Table 2. Relationship of pH and temperature to fruiting, expressed in percentage of cultures forming sporangia. Asterisk (\*) indicates attempted fruiting.

Tempera- ture (C.)	3.0	4.0	5.0	6.0	7.0	8.0	No. of cul- tures
35.0°	***						157
32.5°	68.0	66.0	***				207
30.0°	87.8	80.0	47.2	***			175
27.5°	100.0	96.1	89.7	77.3			146
25.0°	100.0	100.0	93.3	60.0	13.3		105
21.0°	100.0	100.0	100.0	100,0	60.0	55.0	110

attempted fruiting did occur at pH 5.0. The average time required for fruiting at pH 3.0 was 85.5 hours as compared with 67.6 hours at pH 4.0. Spo-

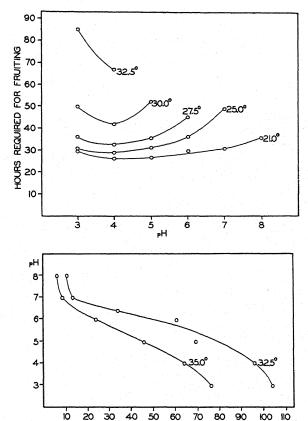


Fig. 1 (above). Graphical representation of the interrelationships of the factors of pH and temperature in their effects upon fruiting.

HOURS REQUIRED FOR KILLING

Fig. 2 (below). The effect of pH upon rate of killing of plasmodia maintained at high temperatures.

rangia appeared in 68 per cent of the cultures at pH 3.0, in 66 per cent at pH 4.0. Since this temperature is definitely lethal except at the two lowest pH values, a record was kept of the length of survival of the various cultures in order to determine if any relationship existed between pH and hours required for killing. The curve of killing obtained from these

Table 3. Relationship of pH and temperature to fruiting, expressed in hours required for fruiting.

Temperature	pH						
(C.)	3.0	4.0	5.0	6.0	7.0	8.0	No. of cultures
32.5°	85.5±2.04	67.6±2.49					38
30.0°	$50.0 \pm 0.58$	$42.4\pm0.58$	$52.4 \pm 1.40$				77
27.5°	$36.1 \pm 0.89$	$32.6 \pm 0.57$	$35.8 \pm 0.61$	$45.5 \pm 2.23$			114
25.0°	$30.3 \pm 0.44$	$29.2 \pm 0.12$	$31.3 \pm 0.38$	$34.9 \pm 1.67$	$49.0 \pm 1.01$		63
21.0°	$30.1 \pm 1.11$	$26.4 \pm 0.61$	$26.5 \pm 0.66$	$29.3 \pm 0.47$	$30.7 \pm 0.60$	$33.1 \pm 0.37$	100

Table 4. Relationship between pH and hours required for killing at high temperatures.

			pH			
Temperature (C.)	3.0	4.0	5.0	6.0	7.0	8.0
Series VI, T = 35.0° Series V, T = 32.5°	75.9±1.02 104.1±3.35	64.6±1.15 95.9±4.43	45.6±3.69 69.2±1.76	23.8±1.20 60.5±3.57	$8.4\pm0.25$ $12.8\pm0.71$	$6.4\pm0.12$ $10.6\pm0.54$

figures is shown in figure 2; data for the two lowest values were obtained from the 32 per cent that were killed at pH 3.0 and the 34 per cent that were killed at pH 4.0. A plasmodium was judged dead when the plasmodial veins began to lose their identity and the color changed from yellow to reddish-brown. In general it may be said that, within the range tested, the higher the pH the quicker plasmodia are killed. In no case did sclerotia formation result from such treatment. Plasmodia of this species obviously can stand somewhat higher temperatures than some other species, since Klebs (1900, p. 138) reported that plasmodia of Didymium difforme (Pers.) Duby form sclerotia at 25°-26°C. but decomposed at 30°C. Either D. difforme is less tolerant of higher temperatures than is P. polycephalum or else the medium upon which Klebs grew his plasmodia was so basic that they were more susceptible. The first hypothesis is the more feasible, because in the present work high pH combined with high temperature did not induce the formation of sclerotia.

Series VI. Temp. = 35.0°C. (157 cultures).—At this temperature fruiting could not be obtained, regardless of the pH at which the cultures were maintained. It is true that attempted fruiting did occur at pH 3.0, but, as in other cases of this nature, no sporangia were ever matured. Killing of plasmodia followed the same general pattern as at 32.5°. Numerical data for each series are presented in table 4, while curves derived from these data are shown in figure 2.

EFFECT OF PH UPON FRUITING IN DARK.—In an earlier paper by the writer (Gray, 1938) it was stated that in general yellow-pigmented plasmodia require light in order to complete their life cycles. This is particularly true for fed cultures, where fruiting without light occurs rarely. The incidence of fruiting of starved cultures in the dark was higher than in fed cultures, so the possibility existed that

the necessity of light for fruiting might be dispensed with in starved cultures if such cultures were placed in an environment where all the other factors were favorable for fruiting. It is to be noted that throughout all the preceding series, a higher percentage of cultures formed fruiting bodies at pH 3.0 than at the higher values; therefore cultures were conducted at this pH in the dark at various temperatures in order to determine if light could be dispensed with. Checks were maintained in moist chambers containing distilled water. The results of these studies are presented in table 5.

From the results of table 5, we can safely conclude that even in starved cultures light cannot be entirely dispensed with by lowering the pH to 3.0, which value produced fruiting in the greatest percentage of cultures in the light. It would seem that in the complete absence of light, high acidity has a definitely unfavorable effect, because at 25.0°C. 13 per cent fruiting was obtained in cultures containing only distilled water, while only 8.2 per cent fruiting was obtained with cultures maintained at pH 3.0. Perhaps the statement that light is a necessary factor may be considered somewhat broad in view of the fact that some fruitings do occur in the complete absence of light; however, a comparison of the figures in table 5 yields the general picture that light is the important factor.

Since, within the range tested, higher temperatures produced unfavorable effects in so far as percentage fruiting and hours required for fruiting are concerned, the possibility existed that perhaps lower temperatures might produce effects that would overcome the necessity of light for fruiting. Cultures were then placed in the dark in a refrigeration room where the temperature varied from 8°-12°C. One or two cultures were removed every twenty-four hours and placed in light at a temperature of 25.0°C. in order to determine if they were alive; this was judged by whether or not streaming was resumed.

Table 5. The effect of pH and temperature upon percentage fruiting in light and in dark. Figures in parentheses indicate the number of cultures in a series.

Temperature	Medium used in moist chamber	Light	Dark
25.0°	Distilled water Buffer at pH 3.0	96.0% (100) 100.0% (100)	13.0% (100) 8.2% (85)
80.0°	Distilled water Buffer at pH 3.0	87.8% (30)	18.0% (50) 3.3% (25)
85.0°	Distilled water Buffer at pH 3.0	0.0% (35) 0.0% (40)	0.0% (100) 0.0% (20)

Plasmodia persisted for considerable periods at 8°-12° but eventually succumbed without fruiting. The rate of killing with respect to pH follows the same general pattern as at the higher temperatures, as may be seen in table 6.

Table 6. The relation of pH to rate of killing of plasmodia at 8°-12°C.

	Days required	
pH	for killing	No. of cultures
3.0	16	35
6.0	10	20
8.0	2	20

Various workers have pointed out that low temperatures induce formation of sclerotia. For example, Klebs (1900) and Constantineanu (1906) both state that D. squamulosum encysts at 1°-2°C., and the latter worker also states that the same is true for Badhamia macrocarpa (Ces.) Rost. DeBary (1864) makes the general statement that a decrease in temperature promotes sclerotia formation. No sclerotia formation was observed in the present work with P. polycephalum, although 241 cultures were involved in the experiments conducted at 8°-12°C. It is true that many plasmodia clumped into a solid mass of protoplasm at this temperature, but there was no apparent loss of water, and certainly these clumps could not be termed sclerotia.

Discussion.—From the foregoing experiments which involved observations on 2,030 different cultures, we may arrive at several general conclusions. First, we may set the optimum temperature for Physarum polycephalum at 20°-21°C., although more will be said about this later. Howard set the optimum for the species at 20°-26°C., but the writer has found that a higher percentage fruiting occurred at 21.0°C. than at higher temperatures; therefore the optimum is set between the lowest value given by Howard and the lowest tested by the writer. Very little difference occurs in length of time required for fruiting at 21°, regardless of pH value, as is shown in the curve of fruiting in figure 1; it will be noted that the curve for this temperature approaches a straight line. The highest temperature at which fruiting may occur is between 32.5° and 35.0°C. Temperatures as high as these are for the most part lethal, and there is a definite relationship between degree of acidity and length of life at a lethal temperature: the lower the pH (between 3.0 and 8.0) the longer the survival. At 35.0° plasmodia in moist chambers, in which the buffer solution is at pH 3.0, survive on the average for three days or more, while similar cultures at pH 8.0 are killed in seven hours

Concerning the relationship of pH to temperature in regard to fruiting, it may be said that the higher the temperature, the lower the pH must be in order to permit sporangia formation. At 32.5° the pH of the solution in the moist chambers must be as low as pH 4.0 or no fruiting will result; at 27.5°, the pH

must be 6.0 or lower, while at 21.0°, fruiting will occur at all pH values up to and including pH 8.0.

In the range tested, temperature remaining constant, the lower the pH the greater the chances are that a plasmodium will fruit as is evidenced by the results in table 2, which lists the percentage of cultures fruiting at various pH values over the temperature range examined. With pH a constant factor, the number of hours required for fruiting varies directly with temperature: the lower the temperature the fewer the number of hours required for sporangia formation.

In all the above series where fruiting was obtained, the least time was required for this process at pH 4.0; therefore we may conclude that the optimum pH for fruiting, with respect to time required, is somewhere near this value. However, the optimum pH for fruiting, with respect to percentage of cultures forming sporangia, was at pH 3.0 (with the exception of Series I and Series II, in which series 100 per cent fruiting was obtained at several of the lower pH values). Therefore, when an optimum pH value is stated, the criterion by which this is judged must be defined, since at one value fruiting is accomplished in the least amount of time, whereas at another value the chances that a plasmodium will fruit are greater. When stating an optimum temperature value, one should be careful that pH value has also been taken into account.

No evidence is obtained from the foregoing studies that sclerotia formation is favored by either high or low temperatures, provided the substratum is moist. Quite possibly there is an optimum temperature for formation of sclerotia, but it would seem that this process is more dependent upon proper

desiccation of the plasmodium.

The fact that higher temperatures (27.5° and above) bring about the production of sessile or short-stipitate sporangia within a species in which the fruiting bodies are normally markedly stipitate might cause one to wonder if perhaps some of the varieties and species now named might not be forms of other species which show variations because of changes in environmental factors. The work of Skupienski (1930) would tend to bear out this conjecture, although many more observations must be made before any definite conclusions are drawn.

### SUMMARY

The interrelationships of the factors of temperature and pH upon the fruiting of *Physarum polycephalum* Schw. have been studied, and from data derived from observations made on 2,030 cultures, the following conclusions are drawn:

The factors of pH and temperature are closely related and interdependent.

The higher the temperature at which a plasmodium is maintained, the greater the acidity must be in order to produce fruiting.

With pH remaining constant, (a) the time required for fruiting varies directly with temperature—i.e., the higher the temperature the longer the time

required for fruiting; (b) the percentage of cultures producing fruiting bodies varies inversely with temperature—i.e., the higher the temperature, the smaller the percentage fruiting.

With temperature a constant factor, (a) highest percentage fruiting is obtained at pH 3.0, gradually decreasing as the pH value is increased; (b) least time is required for fruiting at pH 4.0.

Maximum temperature at which P. polycephalum will fruit is between 32.5° and 35.0°C.

Length of survival of plasmodia at high temperatures is dependent upon pH value and varies in-

versely with it. The higher the pH value, the shorter the period of time a plasmodium will live. This same relationship exists for plasmodia maintained at low temperatures (8°-12°C.).

Under the conditions of the above experiments, sclerotia cannot be produced at high temperatures (32.5°-35.0°) nor at low temperatures (8°-12°).

Generally speaking, the necessity of light for fruiting cannot be dispensed with by increasing acidity nor by lowering the temperature.

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## PLANTS USED BY THE ESKIMO OF THE NORTHERN BERING SEA AND ARCTIC REGIONS OF ALASKA <sup>1</sup>

### J. P. Anderson

During the summer of 1938, through the kindness of the U. S. Coast Guard, the writer was able to visit the Eskimo villages of Northern Bering Sea and Arctic Alaska. He wishes to express his sincere thanks to the officers and crew of the cutter "Northland," on which the cruise was made, and especially to Commander F. A. Zeusler, master of the vessel, and to Lieutenant Commander R. C. Sarrat, executive officer. The writer's chief object was to study the flora of the region and to collect herbarium specimens, of which some 2,100 numbers were secured; at the suggestion of Commander Zeusler, however, some inquiries were made regarding the plants used by the Eskimo, especially those used for food.

The information here presented was obtained to some extent from white school teachers and other white persons resident in the region, but for the most part direct from the Eskimos themselves. The time devoted to the work was very limited, and the subject is worthy of further study. The following notes, though incomplete, may, however, be of interest and may aid anyone wishing to study the subject further.

1 Received for publication September 1, 1939.

The diet of the Eskimo is almost exclusively of animal origin. The total portion that is directly vegetable is very small. The food plants growing in the vicinity of the villages indicated that but little had been gathered. Some exceptions to this rule are found in the Norton Bay region where gardens are grown to some extent, especially at Unalakleet where the Missionary has induced the Eskimo to grow fine gardens with a surplus for sale to boats and for shipment to the market at Nome.

Considering the small amount actually consumed, the number of native species used is surprisingly large; not all the plants listed here, however, are used everywhere they occur, and in some villages only a few seem to be used at all. A more detailed study would be desirable.

Various methods are employed in preparing the plant material for consumption. Some is eaten raw the same as we eat lettuce and celery. Some is simply scalded. Another method is to use either scalding or cold water and then allow the material to ferment, the preparation being ready for consumption when the proper stage of fermentation is reached. They

call this "souring" and sometimes add sugar to the soured material. Ordinarily the material is also boiled.

One of the commonest methods of use is to immerse the plant material in oil. In this way it may be preserved for winter use. On St. Lawrence Island whale blubber is mostly used, while on the mainland the Eskimo frequently uses seal oil; reindeer tallow may be used at times for the same purpose. Oil as used in this paper refers generally to seal oil.

All berries are eaten fresh, and most kinds are also preserved in oil. To a limited extent the Eskimo is learning the white man's methods, and there is a

tendency away from the old habits.

Among the monocotyledons, products of three species are consumed. The enlarged farinaceous bases of a sedge, Carex sp., are called mouse food from the custom of robbing the nest of field mice (Microtis), which gather them for winter food. In some places fish is placed in the mouse nests so that the mice may live through the winter and be able to store a new supply of the sedge the following year. Allium sibiricum L. is prepared by scalding. This species is closely related to the cultivated chives. The seeds of Iris setosa Pall. are roasted, ground, and used as coffee at a few places.

Several species of willow grow in every locality visited, but only one, Salix pulchra Cham., seems to be utilized as food. This species grows scarcely a foot high at Barrow, but the vigor increases southward so that it may attain a height of 6-8 feet at the mouth of the Yukon. The young shoots and catkins are used fresh and in seal oil and may be stored in the oil for winter use. The bark is also peeled off and

the cambium scraped off and eaten.

Alder (Alnus frutica Rupr.) is not secured for food, but the bark is used for dyeing reindeer skins.

Two of the Eskimo's most important food plants

belong to the Polygonaceae. They are Oxyria digyna (L.) Hill. and Rumex arcticus Trautv. The leaves are eaten fresh, soured, boiled, or in oil. The root of Rumex is also utilized, and it is probable that in some localities other species of Rumex than arcticus

are used.

The fleshy taproots of Claytonia acutifolia of the Portulacaceae are used on King Island and a few other places. The leaves of Ammodenia peploides major Hook, a fleshy beach plant belonging to the Caryopluylaceae, and of Rhodiola integrifolia Raf., a succulent of the family Crassulaceae occurring in rocky places, are eaten fresh, soured, or in oil. In some villages one was used but not the other, while in other villages both were used. The roots of Rhodiola are also sometimes procured.

Three species belonging to the Ranunculaceae are utilized. Anemone narcissiflora L. on King Island is used as we use cress and also soured or prepared in oil. Prepared in oil together with other salad greens and beaten to a creamy consistency, it is called cocpotac. This is much relished and when frozen is claimed to be the original Eskimo ice cream. It also has ceremonial significance. In some villages the leaves of Caltha asarifolia DC. are consumed fresh.

Rootstocks of Ranunculus Pallasii are used but become bitter when leaves develop.

Saxifrages are well represented in the flora of the far north, and one type, Saxifrage punctata L., is widely used by the Eskimo. It occurs not in the typical form, but it is represented by geographical races. The succulent leaves are eaten fresh or in oil. They may be preserved for long periods in oil.

Red currants (Ribes triste Pall.) are used where they occur in the Norton Sound region and further

inland northward.

Of the Rosaceae, the dried leaves of Potentilla fruticosa L. are used for tea at Nome. Rubus arcticus L., which intergrades with Rubus acaulis Michx., produces a red berry of very good quality which is here often called strawberry. The most widely distributed of this group of berries, however, is the Cloudberry or Baked-apple berry, Rubus Chamaemorus L., usually but improperly called salmonberry by the Eskimo.

The roots of the leguminous plants of the genus *Hedysarum* are procured. These become rather fleshy and farinaceous in the fall and are used the

same as potatoes.

The seeds of the Beach Pea, Lathyrus maritimus, are roasted and employed in making coffee.

The crowberry, Empetrum nigrum L., is very common, and the fruit is used to a limited extent and

often called Blackberry.

Young shoots of Fireweed (*Epilobium angustifo-lium L.*) are gathered and boiled, usually mixed with *Rumex*. If bacon is added, the dish is said to be delicious.

A species of Mares-tail, *Hippuris tetraphylla* L., furnishes small leaves that are eaten as greens when gathered while young.

At least two umbelliferous plants are used. Ligusticum hultenii Fern. is cut, mixed with fish, and

boiled for winter use. Coelopleurum gmelini (DC.) Ledeb. is used as the white man uses celery.

Bunchberries, Cornus canadensis L. and Cornus suecica L., are often gathered and mixed with other berries.

The Heather family furnishes several species that are utilized by the Eskimo. Cassiope tetragona (L.) D. Don is not a food plant but together with mosses and lichens is gathered for insulating the houses against cold. The Alpine Bearberry, Arctostaphylos alpina (L.) Spreng., is common but is not utilized to the same degree as some of the other berries. The blueberry, Vaccinium uliginosum L., mostly the variety alpinum Bigel., is one of the most important berries. At some Arctic villages the Eskimo must go up the rivers to find it. At Wainwright it is said to grow four miles inland. The Lowbush or Mountain cranberry, Vaccinium vitis-idea minus Lodd, is found on the tundra clear up to Barrow.

Labrador tea, Ledum decumbens (Ait.) Lodd, occurs all over the tundra and is generally used for tea. It is also supposed to have medicinal value. In some places the rootstock of Lungwort, Mertensia mari-

tima (L.) S. F. Gray, is eaten.

A species of *Pedicularis*, known to the Eskimo as Bumblebee plant, furnishes a root that is sometimes eaten and leaves that are soured.

Plants of Achillea borealis Bong. are dried and made into an infusion that has medicinal qualities. Artemisia sp. is also dried and used for medicine. It may be prepared as an infusion for colds, or the powdered plant may be made into poultices for injuries or swellings.

Sweet Coltsfoot, *Petasite frigida* (L.) Fries, is gathered as greens but to a limited extent only.

Dandelion, *Taraxicum* spp., is used in some places. The leaves are scalded.

An infusion made of the needles of White Spruce, *Picea canadensis* (Mill.) B.S.P., is used as medicine for all purposes. The resin is applied to wounds or chewed for pleasure. Its distribution is limited to Norton Sound and inland regions.

Mushrooms are reported as being eaten raw by the natives of St. Michael.

In addition to the above, two or three species were described to the writer which he was unable to locate.

Eskimo Names.—The writer is no linguist, and when he asked the Eskimos their native names for the plants used he found it very hard to find the proper letters to exactly represent the sounds given. The Eskimos of the region speak the same general language, but the dialect varies from village to village, and the region covered is large. In a few cases different names seemed to be used. Also different names are used for various parts of the same plant or for the same part prepared in different ways. Below are a few of these Eskimo names.

### SUMMARY

During the summer of 1938 the writer made a voyage to the Northern Bering Sea and Arctic coasts of Alaska on the U. S. S. "Northland," C. G., on a botanizing trip. Some inquiry was made regarding the use of native plants by the Eskimo; a list is given of 40 seed-bearing plants used by them, mostly for food.

JUNEAU, ALASKA

Scientific name	Eskimo name
Allium sibiricum L	Patitak
Ammodenia peploides major	
Hook	Achaclook
Anemone narcissiflora L	Cocpotac
Arctostaphylos alpina (L.)	
Spreng	Gubluks
Artemisia sp	Sugrit
Carex sp	Bitnix, Kakkot, Puknuk
Cassiope tetragona (L.) D.	,
Don	Kolwerie
Claytonia acutifolia	Koactet, Oackshak
Coelopleurum gmelini (DC.)	
Ledeb	Egoosick
Empetrum nigrum L	Aluit, Bonak
Hedysarum spp	Muchoo
Hippuris tetraphylla L.f	Dookyook
Ledum decumbens (Ait.)	DOOKYOOK
	Dolokost Trees
Lodd (I ) Hill	Delakeet, Iyoo
Oxyria digyna (L.) Hill	Kolnick, Konghuit, Kon-
	holic, Koongalik, Ko-
프레이트 (1984년 - 1984년 -	wolnyok
Pedicularis spp	Ungooigek
Petasites frigida (L.) Fries	Kangwak, Komgwak
Picea canadensis (Mill.)	
B.S.P. Gum	Goochuchglook
Ranunculus pallasii	Kabootie
Rhodiola integrifolia Raf	Eluaklak, Nonavook
Rhodiola integrifolia Raf.,	
Roots	Okveyok
Rubus arcticus L	Beyouwachock
Rubus chamaemorus L	Akpik, Atpit, Epik
Rumex arcticus Trautv.,	
Leaf	Aloukut, Alwaruk, Aska-
	kook, Kiblegrat
Rumex arcticus Trautv.,	,
Root	Kagankuk, Kohlkleguk
Salix pulchra Cham., Cam-	gan
bium	Ahuatabawak
Salix pulchra Cham., Catkins	Ahfelak
Salix pulchra Cham., Shoots.	Chooiya, Kokonick
Saxifraga punctata L	Amslokruk, Asezet, Aziu-
Sandy aga pancoula 11	sak
Vaccinium uliginosum L	
Vaccinium vitis-idea minus	Asievak, Asievet
	77:
Lodd	Kipmingwak, Kopnut, Pornock

# CHROMOSOME COMPLEMENTS OF FIVE SPECIES OF POA WITH AN ANALYSIS OF VARIATION IN POA PRATENSIS <sup>1</sup>

William L. Brown

Grass investigations in the past have dealt primarily with the taxonomic aspect. Recently, however, turf culture and soil conservation have created a need for a more thorough knowledge of the genetic constitution and interrelationships of the plants employed. This study, primarily concerned with variation in *Poa pratensis*, attempts to extend investigations beyond the realm of comparative morphology and to correlate with morphology evidence gleaned from cytological studies. Such a procedure seems necessary in order to arrive at a clearer understanding of the entities involved.

The genus *Poa*, considered usually as one of the more important groups of economic grasses, presents many difficulties from the standpoint of taxonomy and phylogeny. These are due to a number of factors, particularly the large number of species, wide distribution, and extensive cultivation. The taxonomic difficulty is evidenced by the disagreement regarding the position of several species within the genus, which is itself rather loosely held together by more or less arbitrary characters. Sections are in some cases delimited on vegetative characters, which, to say the least, are extremely variable.

The author wishes to express his appreciation to Dr. Edgar Anderson, under whose direction this work has been done, for suggestions and helpful criticisms offered throughout the course of the investigation. To Mr. R. L. Hensel, the author is grateful for the supply of living plants of *Poa arachnifera* from Texas.

MATERIALS AND METHODS.—Living material for cytological study, for the most part, came from the Missouri Botanical Garden Arboretum, Grav Summit. Missouri, and the Missouri Botanical Garden, St. Louis, Missouri. Plants were selected from well established sod, root tips taken from them, and the plants removed either to the garden nursery or greenhouse, depending upon the time of year at which they were taken from the field. In all cases they were brought into flowering, and herbarium specimens have or will be made from them for permanent records. In addition to the Missouri Botanical Garden plants, material from Green Bank, West Virginia, Schoolcraft, Michigan, Washington, D. C., Vanceburg, Kentucky, and College Station, Texas, has been examined. Plants grown from commercial seed from Mangelsdorf Seed Co. and Palmer Seed Co., St. Louis, Missouri, and Scott Seed Co., Marysville, Ohio, have also been examined. This seed, for the most part, came from the region around Kansas City, Missouri.

Root tips were fixed in La Cour's 2 BD or Navashin's fixative, the first always giving superior reults. Following fixation, the tips were run into paraffin according to the method described by La Cour

<sup>1</sup> Received for publication June 29, 1939.

(1931), then sectioned at  $12-15~\mu$ , and the sections stained with crystal violet. Metaphase plates were drawn with the aid of a camera lucida, after which the chromosomes were counted from the drawing. When there is any doubt as to the exact number of chromosomes present, this has been indicated by  $\pm$  1.

Chromosome numbers of four native species.—While the European species of the genus *Poa* have in the past received considerable attention from cytologists, the native North American species have been almost entirely neglected. Chromosome numbers of four species indigenous to this country are herewith reported for the first time.

Poa Wolfii Scribn. (Gray Summit, Missouri). Ohio to Minnesota and Missouri. 2n = 28. Figure 1.

Poa arachnifera Torr. (College Station, Texas). Southern Kansas to Texas and Arkansas; introduced eastward into South Carolina and Florida; Idaho. 2n = 42. Figure 2.

Poa cuspidata Nutt. (Vanceburg, Kentucky). New Jersey to Ohio, south to Georgia and eastern Tennessee. 2n = 28. Figure 3.

Poa sylvestris A. Gray. (Gray Summit, Missouri). New York to Wisconsin, south to Florida and Texas. 2n = 28. Figure 4.

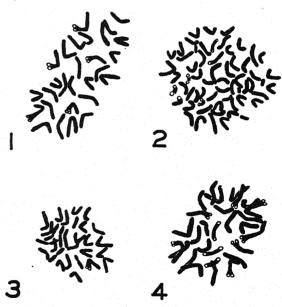


Fig. 1-4. Chromosome complements of four North American species of *Poa*. Metaphase plate, × approx. 2400.—Fig. 1. *Poa Wolfii* Scribn. 2n = 28.—Fig. 2. *P. arachnifera* Torr. 2n = 42.—Fig. 3. *P. cuspidata* Nutt. 2n = 28.—Fig. 4. *P. sylvestris* A. Gray. 2n = 28.

POA PRATENSIS.—History.—A complete taxonomic history of Poa pratensis is given in the recent work of Mecenović (1939). However, the more important treatments which have appeared since 1811

are presented in table 1 and clearly indicate the difference in opinion regarding the taxonomy of the species.

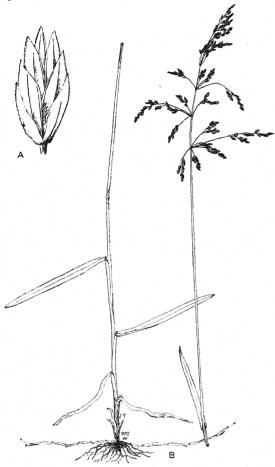


Fig. 5. Composite drawing of *P. pratensis* L. depicting a Series I plant. (A) Spikelet (×8.4). (B) Habit (×.42).

Table 1. Summary of taxonomic treatments of Poa pratensis indicating the degree to which the species has been segregated by various investigators.

Investigator	Number of entities
Gaudin (1811)	7
Döll (1843)	5
Prodr. Fl. Bat. (1851)	9
Andersson (1852)	10
Schur (1885)	8
Ascherson and Graebner (1900)	22
Rouy (1913)	14
Podpěra (1927)	19
Hayek and Markgraf (1932)	11
Jirásek (1934)	21
Jansen and Wachter (1936)	20

Morphological variation within the species.— Morphologically Poa pratensis can roughly be divided into two groups, for convenience referred to here as Series I and II. The first includes most cultivated forms of the species and perhaps some individuals from endemic populations. They are characterized by narrow leaves, averaging 1.5–2 mm. in width, small spikelets, usually three florets per spikelet, coarse, stiff, or wiry roots, and a small number of rhizomes. The second series presents a very different picture morphologically. The leaves are much wider, averaging 3.5–5 mm., and shorter than those of the previous group. The spikelets are larger and the number of florets in each spikelet tend to be greater (4 to 5) than in Series I. Rhizomes are numerous and are longer than in the first named group.

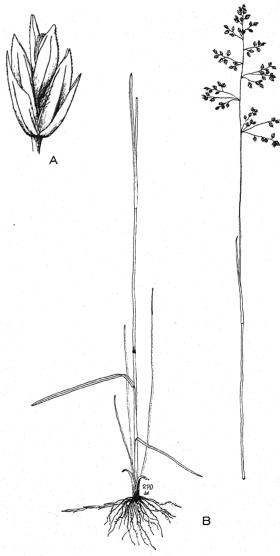


Fig. 6. Composite drawing of *P. pratensis* L. depicting a Series II plant. (A) Spikelet (×6). (B) Habit (×0.3).

The growth of these two series of forms shows striking differences. Series I, when studied in a given area of sod, presents a large number of individual plants growing close together and forming a tightly compacted mass. During fall or winter, few, if any, new rhizomes are developed. Growth is severely checked by even moderate cold, and successful transplanting can be accomplished only under the best possible environmental conditions.

A study of Series II, in a given area of sod, reveals fewer plants. All the individuals, however, are quite vigorous and are spread out so as to cover a larger area than do those of Series I. Numerous actively growing rhizomes are present in fall and winter. Growth is checked only by extreme cold, and the plants can be successfully transplanted even under the most adverse conditions. As was stated above, individuals of *Poa pratensis* can roughly be classified as belonging to one of these groups, but if a large number of plants are examined critically, their division into two groups is very difficult. Studies involving large populations show many individuals which can be classified only as intermediates between the two extremes mentioned. Although it is

difficult to build a series of forms ranging from one extreme to the other, this can be done to some extent. It is true that at least three characters remain more or less constant throughout all forms of the species. These are the presence of a web at the base of the lemma, silky pubescence on the lower half of the marginal nerves, and glabrous intermediate nerves. Neither is the shape of the ligule very variable, but the diversity in other important characters is such as to convince one that it cannot be due to chance alone.

Figures 5 and 6 show in a more exact manner the type of variation under discussion.

Cytology.—The cytological literature of Poa pratensis shows a wide variation in the chromosome numbers reported. The species possesses a basic chromosome number of seven and is highly polyploid. Diploid individuals have not as yet been found. Reported numbers range from tetraploids to

Table 2. Summary of chromosome numbers previously reported for Poa pratensis. Descriptions of plants were taken from publications in which the numbers were reported.

Origin	Morphology	2n	Investigator
weden		50	Åkerberg
weden		51	Åkerberg
weden	· · · · · · · · · · · · · · · · · · ·	50	Åkerberg
weden		48	Åkerberg
Sweden		49	Åkerberg
weden		66	Åkerberg
weden	·	68	Åkerberg
Sweden		74	Åkerberg
weden		49	Åkerberg
weden		47	Åkerberg
weden		52	Akerberg
weden	• • • • • • •	94	Åkerberg
Sweden	•••••	95	Åkerberg
Sweden		53	Åkerberg
Sweden		54	Åkerberg
Sweden		55	Åkerberg
Sweden		56	Åkerberg
Ottawa, Canada	Uniform, upright, early flowering	69	Armstrong
Aberystwyth	Lacking in vigor, leaves		
	waxy, late flowering 86	& $87 \pm 1$	Armstrong
Aberystwyth	Narrow-leaved	$84 \pm 1$	Armstrong
Danish Pasture	Wide-leaved 70	& 50	Armstrong
Sweden	Leaves intermediate	72	Armstrong
Ontario Agricultural College	Wide-leaved, vigorous	54	Armstrong
Ottawa	Variable	64	Armstrong
Commercial Seed	Variable	56	Armstrong
Commercial Seed	Variable 50	& 65	Armstrong
Russia		28	Avdulow
Russia		56	Avdulow
Russia		70	Avdulow
Svalov, Sweden	Constant (no description)	68	Müntzing
Svalov, Sweden		64	Müntzing
Svalov, Sweden		85	Müntzing
Svalov, Sweden	Constant (no description)	75	Müntzing
North Sweden	Constant (no description)	75	Müntzing
North Sweden	(	81-82	
Iceland	Constant (no description)	80-81	Müntzing
	Constant (no description)	49	Müntzing
Japan (?)	·····	70	Nakajima

thirteen-ploids, including varying degrees of aneuploidy.

Stählin (1929) found the somatic chromosome number of P. pratensis to be 56. Avdulow (1931) reported the occurrence of 2n numbers of 28, 56, and 70. Nakajima (1933) lists 70 as the 2n number for P. pratensis. The same year Müntzing, working with Swedish material, gave for eight collections the following somatic numbers: 68,  $\pm$  64,  $\pm$  85,  $\pm$  75, 81–82, and 49. Seven of the eight biotypes in-

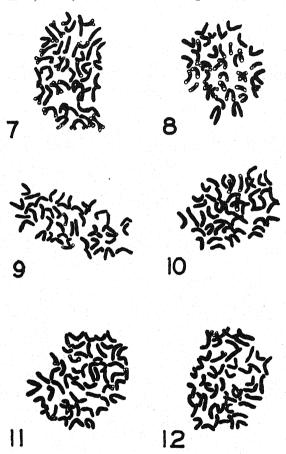


Fig. 7-12. Chromosome complements of six biotypes of  $P.\ pratensis\ L.$  Metaphase plates,  $\times$  approx. 2400.—Fig. 7. Nursery E. D. 2n=45.—Fig. 8. Nursery S. E. B.  $2n=41\pm 1$ .—Fig. 9. Nursery N. B. 2n=45.—Fig. 10. Nursery E. A.  $2n=50\pm 1$ .—Fig. 11. Cabin C. 2n=56.—Fig. 12. Nursery N. A. 2n=49.

vestigated by Müntzing are shown to have aneuploid numbers. In a study of seed formation in P. pratensis, Åkerberg (1936) lists chromosome numbers ranging from 47 to 95. Armstrong (1937) determined the chromosome number of nineteen plants from nine sources, including commercial seed and supposedly indigenous sod. Nine of his plants are shown to have euploid and ten to have aneuploid numbers—namely, 50,  $70\pm1$ , 72, 54,  $86\pm1$ ,  $87\pm1$ ,  $84\pm1$ ,  $69\pm1$ , 70, 64, 56, and 65. In all the works cited above (excepting Armstrong) little

if any mention is made of the morphological characters of the plants examined. This is unfortunate, since chromosome numbers without accompanying morphological data are of little significance to either the systematist or the cytologist.

Chromosome numbers as reported in previous cytological works are summarized in table 2.

Observations.—The twelve collections of pratensis studied, with their somatic chromosome numbers and outstanding morphological characters, are listed in table 3. The localities from which the material has been collected are used as names merely to distinguish the plants.

Following is a description of the plants listed in table 3.

Cabin C. An octoploid: Leaves averaging 2-2.5 mm. in width. Spikelets containing three small florets. Rhizomes few. 2n = 56. Series I.

Cabin G. A plant hardly distinguishable from Cabin C, except for its chromosome number.  $2n = 56 \pm 1$ . Series I.

Nursery E. C. A form with narrow leaves which average 2 mm. in width. Leaves somewhat more involute than those of Cabin C. or G. Rhizomes few. 2n = 56. Series I.

Nursery E. A. An intermediate between Series I and II.  $2n = 50 \pm 1$ .

Nursery N. A. 2n = 49. Series I.

Nursery E. D. A wide-leaved an euploid with numerous rhizomes. Extremely vigorous. Leaves lacking the dark green color of most forms. 2n = 45. Series II.

Alleghany. Intermediate between Series I and II. Leaves averaging 2-2.75 mm. in width, 2n = 56.

Nursery N. B. 2n = 45. Series II.

Nursery S. E. B. A wide-leaved type, leaves averaging 3.5 to 4 mm. in width. Rhizomes numerous.  $2n=41\pm1$ . Series II.

Nursery S. X. The extreme in this direction. Leaves averaging 4 mm. in width. Single plant may possess as many as sixty rhizomes. 2n = 42. Series II.

Helms. Leaves narrow, averaging 1.5 mm. in width. Spikelets containing three florets. Rhizomes few. 2n = 64. Series I.

Schoolcraft. 2n = 56. Series I.

Table 3. Summary of chromosome numbers of Poa pratensis reported in this investigation.

Plant	Source	Series	2n
Cabin C	MBGAa	İ	56
Cabin G	MBGA	I	56±1
Nursery E. C	MBGb	I	56
Nursery E. A	MBG	Intermediate	50±1
Nursery N. A	MBG	I	49
Nursery E. D	MBG	II	45
Alleghany	Alleghany M	t.,	
	8 mi. E.		
	Green Ban	k.	
	W. Va.	Intermediate	56
Nursery N. B	MBG	II	45
Nursery S. E. B.	MBG	II	41±1
Nursery S. X	MBG	II	42
Helms	MBGA	I	64
Schoolcraft	Schoolcraft,		
	Mich.	I	56

<sup>\*</sup> Missouri Botanical Garden Arboretum, Gray Summit, Missouri

b Missouri Botanical Garden, St. Louis, Missouri.

It will be noted that five of the twelve collections reported are aneuploid, a fact not in absolute agreement with previous cytological work. Müntzing, for instance, found aneuploid numbers in seven of the eight biotypes investigated by him. This variation in the extent of occurrence of aneuploid forms in different parts of the world may in some degree account for the diversity of opinion concerning the extent of apomixis within the species.

Müntzing's evidence for the occurrence of apomixis in this species is based on the cytological and morphological constancy of aneuploid forms. Although these facts are suggestive of apomixis, the evidence is of course indirect. If, however, the percentage of aneuploidy present is used as a basis upon which to work, the present investigation would indicate that apomixis in *Poa pratensis* may not be as common a phenomenon as is often supposed. The problem can be solved satisfactorily only through critical progeny analyses and embryological studies.

It has been found from the plants examined and from the cytological literature that octoploidy is more frequent than any other degree of polyploidy (fig. 13). This may be at least partially explained by the occurrence of octoploidy in most cultivated forms. Degrees of polploidy beyond thirteen-ploids have not been observed in this study, nor have the lower polyploid forms (tetraploids and pentaploids) been found. The lowest 2n number encountered is a near hexaploid,  $41 \pm 1$ , while the highest number is 64.

Although the chromosome morphology of these forms has not been studied intensively, there is considerable variation in chromosome length. Attachment constrictions are usually median or sub-median, regardless of the length of the chromosome involved (fig. 7–12). No satellited chromosomes have been found.

Correlation of cytology and morphology.-Leaves, spikelets, and rhizomes all possess the range of variability characteristic of the species, and since leaves and rhizomes can be observed throughout the year, they have been selected as the principal morphological tools with which to work. The leaves of mature plants of pratensis vary in width from 1.5 to 4.5 mm. Although length of leaves has not been as carefully studied as has leaf width, the wide leaves are usually shorter than the narrow ones. Inflorescences are divided easily into two groups: the first possesses a relatively narrow panicle, small spikelets, each spikelet containing 2 to 3 (usually 3) florets; the second type characterized by a wide, open panicle with large spikelets, each spikelet containing 4 to 6 florets.

Rhizomes within the group show a difference only in number and length, but it is not due to environment. Plants selected from various localities and grown under identical<sup>2</sup> conditions vary extremely in the number of rhizomes produced in a one-year period. Some plants under ordinary conditions will

<sup>2</sup> With the exception of any soil heterogeneity which might be present within a given plot.

produce in one year as many as sixty new rhizomes, others grown in the same plot will produce only ten or twelve. All plants, of course, cannot be separated into groups producing approximately sixty or approximately ten new rhizomes in one year. Numerous intermediate forms are found. Variation in a group of plants is in itself of little significance, but the manner of variation is quite important, and it is that point which should be emphasized in this particular case.

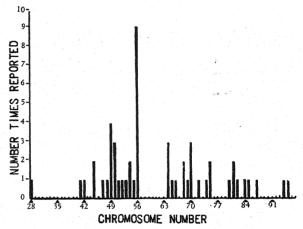


Fig. 13. Distribution of chromosome numbers in P. pratensis L.

Variation in *P. pratensis* is not at random, but there is a strong tendency for morphological characters to remain together in groups. For example, one finds in combination with wide leaves wide panicle, large spikelets, tendency toward an increased number of florets in each spikelet, and long and numerous rhizomes. The opposite characters tend to be carried in combination with narrow leaves. In a study of herbarium specimens composed of plants representing the entire range of *P. pratensis* in the United States, there was found to be a significant correlation (fig. 14) between leaf-width and number of florets in each spikelet. In this study the leaf-width and floret-number for each plant represent an average of at least six counts.

Excellent facilities for a study of the relationship between leaf-width and rhizome-number were offered by plants growing in the nursery, Rhizome counts and measurements of leaf-width were made on these plants in the spring of 1939. Due to the fact that in this climate P. pratensis rhizomes develop almost entirely during September to November, it was possible at that time to determine with fair accuracy the number of new rhizomes developed within one year. Although the small number of plants used here reduces the significance of the correlation, there still is shown to be a definite relationship (fig. 15) between leaf-width and number of rhizomes present. This, in addition to the evidence mentioned earlier, seems to be a sufficient basis for establishing the fact that characters, although variable, do not segregate at random but remain together in groups. One may also assume that this variation is regulated by two distinct genomes which, for convenience, may be designated as genome A and genome B. The action of these will be discussed later.

An explanation of the above may be found in the cytology of the plants involved, which is no less variable or complex than is the morphology. At least

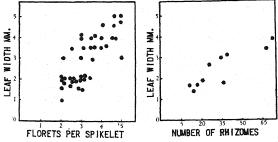


Fig. 14 (left). Analysis of forty plants showing the relationship between leaf-width and number of florets per spikelet.

Fig. 15 (right). Analysis of ten plants showing the relationship between leaf-width and rhizome number.

twenty-eight chromosome numbers have been reported for the species, a range of counts fairly high for a group of plants possessing a basic chromosome number. The cytological evidence at hand suggests the operation of two processes which, when working in combination, may account for the extreme variation in this plant. These are (1) polyploidy and (2) apomixis.

Since polyploidy tends to increase the potential range of species variation, it is logical to expect a wider range of variability in P. pratensis, which has a polyploid range of 4n to 13n, than in a species of less cytological complexity. The type of polyploidy (allo- or autopolyploidy) operating within P. pratensis has not as yet been determined, and the direct evidence will have to come from a study of chromosome association at meiosis. On the other hand, critical observation of the variants enables one to form an hypothesis regarding the type of polyploidy involved. Were only autopolyploidy present, one would expect to find changes in degree only. Since there has been shown in pratensis changes not only in degree but also in form, it seems justifiable to suggest the presence of at least some allopolyploidy.

Another point which supports to some extent the theory for an alloploid origin of *P. pratensis* is the presence of apomixis, a phenomenon often associated with hybridization. It seems probable that polyploidy, whether it be allopolyploidy, autopolyploidy, or a combination of the two, is the most important factor in the production of variable types within the species. On the other hand, apomixis certainly has made possible the persistence of many forms which, if sexual, would have been partially or entirely eliminated due to difficulties of chromosome pairing at meiosis (Darlington, 1937, Ch. IV). Contributions to our knowledge of apomixis and the development of the caryopsis in *Poa* have been made

by Andersen (1927), Nishimura (1922), Müntzing (1933), Åkerberg (1936), and Kiellander (1937). Despite these works and other papers which have appeared on the subject, the extent of apomixis in *P. pratensis* is still an unsettled question. At present the most conclusive evidence for this phenomenon is the fact that a large number of aneuploid forms exist in any population. If these forms are cytologically and morphologically constant, as has been suggested by other workers, then the evidence, although still not direct, is somewhat more conclusive.

Before offering a specific cytological explanation of the variation in this plant it is necessary to describe any association which may exist between cytology and morphology. Briefly it is as follows. Plants with wide leaves, large spikelets, and many rhizomes are relatively low polyploids, Plants with narrow leaves, small spikelets, and few rhizomes, on the other hand, are high polyploids. Those plants whose morphological characters tend to place them as intermediates between Series I and Series II have chromosome numbers intermediate between high and low polyploids. In *P. pratensis* the optimum for chromosome increase beyond which there is a decided decline in vigor is near the hexaploid condition.

Using the evidence cited above, the following hypothesis might be offered as an explanation of the processes continually at work within the germ-plasm and which are in turn directly responsible for the extreme variation as expressed in the phenotype. It has been previously mentioned that the germ-plasm of pratensis is apparently composed of two genomes which, as expressed in the morphology of the plant, are found to exert entirely different influences. For instance, one genome (A) may carry characters for wide leaves, large spikelets, and long rhizomes; the other (B) may be capable of producing narrow

	2n	Genome balance
Cabin C	56	AAAA/BBBB
Cabin G	56±1	AAAA/BBBB
Nursery E. C	56	AAAA/BBBB
Nursery E. A	50±1	AAAA/BBB
Nursery N. A	49	AAA/BBBB
Nursery E. D	45	AAAA/BB+3
Alleghany	56	AAAA/BBBB
Nursery N. B	45	AAAA/BB+3
Nursery S. E. B	$41 \pm 1$	AAAA/BB
Nursery S. X	42	AAAA/BB
Helms	64	AAAA/BBBBB+
Schoolcraft	56	AAAA/BBBB

leaves, small spikelets, and short rhizomes. It is logical to assume that the morphology of any given plant is determined by the balance between the two kinds of genomes composing its germ-plasm. A plant of the constitution AAAA/BBBB would be expected to be a more extreme example in the direction of Series I than a plant whose germ-plasm contained AAAA/BB. In this connection it may be expedient to list the hypothetical genome balance of all the plants included in table 3.

It is shown in the list above, as well as in figure 13, that 56 is the modal chromosome number for the species. It seems most likely that this number has arisen from a cross between two tetraploids of the constitution AAA'A' × BBB'B'. If this is true. one can then assume that the various other forms within the species, with chromosome numbers other than 56, have arisen by a loss or duplication of one or more chromosome sets. Thus 49, the number of next greatest frequency, is likely the result of a loss of one chromosome set, and since for the most part those plants possessing 49 chromosomes are members of Series II, the lost set may be assumed to have belonged to genome B. The number 64 has probably arisen from 56 by a duplication of one genome plus one chromosome. In this instance the extra genome is apparently a member of A, since plants with 64 chromosomes belong to Series I.

Until further study yields more direct evidence this can of course be accepted only as a working hypothesis. The germ-plasm of P. pratensis may contain three or even more genomes, but the evidence at hand does not suggest the presence of more than

In view of the above cytological and genetic evidence the presence of extreme variation in this plant is not surprising. One would expect the tetraploid, hexaploid, octoploid, and decaploid forms to be more or less regular cytologically and thus capable of reproducing themselves sexually. This complex alone would result in no small amount of intra-specific variability. Furthermore, when to this sexual complex are added the various aneuploid forms which are being reproduced asexually through apomixis, it is understood easily why P. pratensis presents such a variety of diverse forms.

According to these observations, the segregation of species, subspecies, varieties, forms, etc., from P. pratensis, a practice well known to European botanists, hardly seems justifiable. It is true that without much difficulty forms which are morphologically different can be found, but it is difficult to understand how such groups can be segregated either specifically or varietally without overlooking intentionally the various intermediate forms.

### SUMMARY

Previously unreported chromosome numbers have been determined for four species of the genus Poa.

A high degree of non-random intra-specific variation is present in Poa pratensis. This is due to polyploidy and aneuploidy.

Chromosome numbers ranging from  $\pm$  41 to 64 are reported for twelve biotypes of pratensis.

The probable occurrence of apomixis and the presence of "group segregation" form a basis for the suggestion of an alloploid origin for the species. An hypothesis, based on the genome balance

within the germ-plasm, is offered as an explanation of the morphological variation within the species.

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# RESPIRATION AND FERMENTATION IN THE CARROT, DAUCUS CAROTA I. RESPIRATION <sup>1</sup>

# Paul B. Marsh and David R. Goddard

ALTHOUGH THERE has been a great deal of research in recent years dealing with the nature of respiration and fermentation in yeast and in animal tissues, higher plants have not been intensively investigated in this regard. It has been the purpose of this study to investigate the nature of respiration and fermentation in the carrot, Daucus carota, and to extend our knowledge concerning the relationship between these two processes. Part I is a report of the experiments on respiration, while Part II, to be published shortly, presents the experiments on fermentation and its relationship to respiration.

Three distinct oxidases involved in plant respiration are now known-cytochrome oxidase, catechol oxidase, and a group of oxidases known as the flavine or vellow enzymes. Cytochrome oxidase (Keilin, 1929) also known as indophenol oxidase or phaeohemin oxidase (Warburg, 1927), is found in veast and is widely distributed in the animal kingdom. It is inactivated by cyanide, azide, and carbon monoxide, the carbon monoxide inhibition being light reversible. Catechol oxidase (Batellei and Stern, 1912; Keilin and Mann, 1938; Kubowitz, 1937), occuring primarily in plants, is inactivated by the same three poisons, but the carbon monoxide inactivation is not light reversible. The flavine enzymes (Theorell, 1937), the first of which was isolated from yeast only seven years ago (Warburg and Christian, 1932), are cyanide-insensitive oxidases, whose prosthetic group is a phosphate ester of vitamin B2 (riboflavine).

In this paper we present evidence for the operation of two different oxidases in the carrot plant. One of these is quite similar to or identical with the cytochrome oxidase and accounts for the major part of the respiration of the carrot root and young leaves. The other, insensitive to poisoning by cyanide, azide, and carbon monoxide, accounts for a fraction of the respiration of roots and young leaves and the entire respiration of mature leaves.

Materials and methods.—Warburg (1928) clearly stated the simple assumption involved in the use of reversible enzyme poisons such as cyanide and carbon monoxide in the study of cell metabolism. A poison of known chemical behavior is assumed to react inside the intact cell with enzymes which contain chemical groups of the same type with which this poison reacts in vitro. Removing the poison or otherwise breaking up the enzyme-poison compound allows the metabolism to return to its original unpoisoned state. By this method we may study respiration and fermentation in the cell under relatively normal conditions.

Carrots of the horticultural variety Nantes Coreless, grown in the university greenhouse, were used for these experiments except where otherwise designated. Some preliminary experiments were made

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on carrots of unknown variety from commercial sources; these carrots are designated "B." Experiments in the fall of 1938 were made on carrots of the variety Nantes Coreless bought from a farmer near Rochester, stored in sand in a cool place and used as needed; these carrots are designated "F." No qualitative differences among these carrots from different sources have been noted in the experiments to be reported, and all direct quantitative comparisons are between carrots from a single source.

Plugs 6 mm. in diameter were cut from the carrot roots with a cork borer and the cortex from the plugs sliced with a razor into sections approximately 0.5 mm. thick. Oxygen consumption and carbon dioxide production of 400–500 mg. wet weight of these slices suspended in 2 ml. of M/60 Sorensen's phosphate buffer of pH 5.9 were measured in a Fenn (1928) microrespirometer at a temperature of  $22 \pm 0.005$ °C.

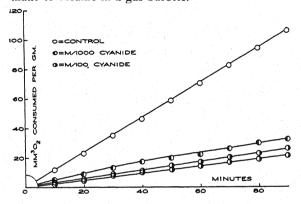
The apparatus used had a capillary volume of 3.0 mm.3 per cm. of length, giving a sensitivity which varied for individual manometers of 1.0 cm. deflection equal to 5.5 to 6.0 mm.3 Readings were taken at five- or ten-minute intervals and the oxygen consumption calculated by multiplying the observed movement of the bubble times the oxygen constant for the particular vessel. The rate of oxygen consumption was usually fairly constant with time, as may be seen, for instance, from the control vessel values in figure 1. Although the rate of oxygen consumption per gram fresh weight per hour varied considerably from one batch of carrots to another, the agreement between samples used in any one experiment was fairly satisfactory. The percentage deviation from the mean of two duplicate controls for a 60-minute interval was calculated for each of ten experiments; the average percentage deviation from the mean was 4.3 per cent.

Respiration of leaf tissue was measured by floating 100 mg. samples on phosphate buffer. All respiratory measurements on leaves were made in a photographic dark room, using a very weak light only during the actual reading of the apparatus. Since large respiratory differences exist between different samples of carrot leaves, the oxygen consumption of unpoisoned leaves was first determined, and then the poisons added, and a second determination made on the same tissue.

The rate of oxygen diffusion into the tissue from air did not limit the rate of root respiration. At 2.5 per cent oxygen the rate of respiration was always below the rate in air, but at 5 per cent there was sometimes a decrease and at other times not. Values for mm. <sup>3</sup>/gm./hr. in air of 93.2, 91.0, 97.8, 76.0, 76.0 were obtained as against corresponding values in the same five experiments of 88.2, 57.0, 75.2, 84.0, and 53.0, respectively, for 5 per cent 0<sub>2</sub>, 95

per cent  $N_2$ . Raising the oxygen percentage to 100 per cent resulted in a slight, but probably not significant, increase in oxygen consumption. Four hundred mg. of roots consumed 49.6 mm.<sup>3</sup> of  $0_2$  in 70 minutes in air and 54.2 mm.<sup>3</sup> in a subsequent equal period of time in pure  $0_2$ .

Acid or alkaline poisons were made up at several times the desired final concentration, neutralized to pH of 5.7–6.0 as determined at a quinhydrone electrode, then diluted with phosphate buffer of pH 5.9 to give the desired concentration. The acidity of the poison-containing suspending media was rechecked as a routine matter after each experiment. In poisoning with cyanide, KOH–KCN mixtures were used in the inset (Krebs, 1935). CO was made by dehydrating formic acid with hot sulfuric acid, was passed through sodium hydroxide solution to remove any traces of formic acid and the gas mixtures made to volume in a gas burette.



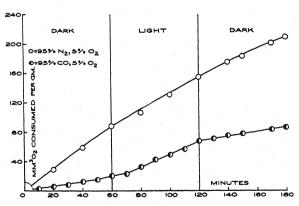


Fig. 1 (above). Inhibition of carrot root respiration by cyanide.

Fig. 2 (below). Light reversal of carbon monoxide inhibition of carrot root respiration.

EXPERIMENTAL RESULTS.—Reversible inhibition of root respiration by cyanide.—The respiration of carrot roots, like that of yeast and muscle, is reversibly inhibited by low concentrations of cyanide, as may be seen from table 1 and figure 1. The respiratory rate of cyanide-poisoned carrots, after an initial period of falling rate, was practically constant with time (see fig. 1). It will be noticed from the table that even with the highest concentrations

of cyanide there remained a small residual respiration. The reversible nature of the inhibition was established by removing the carrots from the cyanide media, washing, and replacing the KOH-KCN inset mixture with KOH. Values of oxygen consumption in cyanide in mm. <sup>3</sup>/gm./hr. of 21.5, 19.8, 23.6, and 27.7 rose to 60.5, 42.1, 67.5, and 95.0, respectively, after washing.

Table 1. Inhibition of root respiration by cyanide.

Molar concentra-		mm.3	mm. $^3 \theta_2/\mathrm{gm}$ .	
tion of cyanide M	nutes	Control	Poisoned	age in- hibition
1.0 × 10 <sup>-5</sup>	80	87.6	80.0	8.7
$1.0 \times 10^{-4}$	50	(87.6)a	46.8	47
$1.0 \times 10^{-4}$	60	74.9	41.7	44
$1.0 \times 10^{-4}$	60	(74.9)	35.8	52
$1.0 \times 10^{-4}$	60	(74.9)	31.9	57
$3.0 \times 10^{-4}$	60	(102)	23.9	77
$3.0 \times 10^{-4}$	60	(102)	24.8	76
$3.0 \times 10^{-4}$	60	101	25.0	75
$8.0 \times 10^{-4}$	60	(102)	11.1	89
$8.0 \times 10^{-4}$	60	(102)	14.4	86
b1.0 × 10⁻³	80	119	31.3	74
b1.0 × 10⁻3	80	(119)	28.0	76
b1.0 × 10⁻³	90	106	32.0	70
b1.0 × 10⁻2	90	(106)	21.2	80
$^{b}1.0 \times 10^{-2}$	90	(106)	26.2	75

<sup>\*</sup> The parentheses indicate use of a single control vessel to determine the percentages of inhibition for several poisoned samples in a single experiment.

<sup>b</sup> Source B.

Light reversible inhibition of root respiration by carbon monoxide.—It was found that carrot root respiration is inhibited by carbon monoxide and that this inhibition may be reversed by light. Light from a bank of Mazda lamps, one 100-watt lamp about ten cm. below each experimental vessel, caused this reversal in each of the ten determinations. A graph of a characteristic experiment is shown in figure 2, while table 2 presents the results of three other experiments.

Warburg (1927) showed that with substrate saturation the ratio of residual respiration/respiration

Table 2. Inhibition of root respiration by carbon monoxide and light reversal of this inhibition. Poisoned = 95 per cent CO, 5 per cent  $0_2$ ; Control = 95 per cent  $N_2$ , 5 per cent  $0_2$ .

$0_2$ consumed in mm.3/gm.				
Experiment		60 min. dark	60 min. light	
1	Poisoned	26,0	64.6	
	Poisoned	25.8	57.3	
	Control	75.2	76.8	
2	Poisoned	18.9	67.1	
	Poisoned	22.4	66.3	
	Control	56.6	79.0	
3	Poisoned	24.3	44.3	
	Poisoned	20.5	62.3	
	Control	63.4	55.6	

inhibited is proportional to  $pO_2/pCO$  and also proportional to a constant, K, expressing the relative affinity of the enzyme for  $O_2$  and CO. Using the equation:  $K = \text{residual respiration pCO/respiration inhibited pO}_2$ , Warburg obtained values for K of from 4.3 to 13 in experiments with yeast. We obtained values for K from our experiments which are quite comparable to Warburg's figures (see table 3). (Warburg points out that although haemoglobin also forms a compound with carbon monoxide, its K is about 0.01.)

Table 3. Relative affinity of the enzyme for oxygen and for carbon monoxide. Sixty minutes in all cases. Poisoned = 95 per cent CO, 5 per cent  $\theta_2$ . Control = 95 per cent  $N_2$ , 5 per cent  $\theta_2$ .

	$0_2$ in mm.3/gm.		Percentage		
F	oisoned	Control	inhibition	K	
	20.7	53	61	12.1	
	20.1	(53) <sup>a</sup>	62	11.6	
	26.0	75.2	65	10.2	
	25.8	(75.2)	65	10.2	
	18.9	56.6	67	9.4	
	22.4	(56.6)	60	12.7	
	20.5	63.4	67.6	9.1	
	24.3	(63.4)	61.6	11.8	

\* The parentheses indicate the use of a single control vessel determination to calculate percentages of inhibition of more than one poisoned sample in the same experiment.

Kempner (1936) has observed light-reversible CO inhibition of respiration in several plant tistues—plum and daffodil pistils and stamens, pine needles, leaves of tobacco, plum and oleander.

Reversible inhibition of root respiration by azide.

—Carrot root respiration, like that of yeast and heart muscle, is inhibited by low concentrations of sodium azide (NaN<sub>3</sub>). However, the percentage inhibition does not increase markedly with increasing acidity of the suspending medium as is the case with yeast and heart muscle (Keilin, 1936). A sixtyminute experiment gave the results shown in table 4.

Table 4. Inhibition of root respiration by azide at different acidities.

- Hamana	$0_2$ in mm.3/gm. in 60 min.				
*******	pН	Control	10-3 M NaN <sub>3</sub>	Inhibition	
	4.5	69.2	13.0	81.2	
	5.1	80.2	19.6	75.6	
	5.7	68.4	19.8	71.0	

The sodium azide inhibition was reversed by washing the carrot slices thoroughly in distilled water. A determination of the normal rate of respiration of three unpoisoned samples was followed by a determination of the respiratory rate in M/1,000 sodium azide; finally these samples were washed in water and the three individual respiratory rates de-

termined again. Results of this experiment are presented in table 5.

Table 5. Reversal of NaN<sub>3</sub> inhibition of respiration. Total time for each determination: 30 minutes. Readings at 5-minute intervals and rates constant. NaN<sub>3</sub> at 10-3 M.

	$\mathrm{mm.^3~0_2/g}$	m./hr.	
Vessel	Unpoisoned	Poisoned	After washing
1	126	30.9	118
2	112	28.2	102
3	98.0	23.6	79.5

Dye reactions.—Since the respiratory inhibitions of carrot roots showed a general similarity to those of yeast, experiments were undertaken on the indophenol oxidase reaction. Yeast and most animal tissues markedly catalyze the oxidation of di-methyl p-phenylene diamine and alpha-naphthol (the Nadi reagent) to indophenol blue by molecular oxygen. This reaction in yeast is completely abolished on killing the yeast by boiling and is sensitive to the same poisons (HCN, H2S, CO with light reversal) as normal yeast metabolism and the oxidation of cytochrome in intact yeast (Keilin, 1929; Meldrum, 1934). The carrot roots gave a weak but positive reaction with the Nadi reagent and unexpectedly gave a positive reaction even after boiling the tissues. This observation led us to measure the oxygen consumption of dead carrots plus dyes.

Table 6. A dye oxidation catalyzed by dead carrots. Source: F.

mm.3 $0_2$ consumed/hr./350 mgs. tissue		
Live carrots:		
Control	26.8; 26.6	
Plus P.P.D.a	47.9; 54.3	
Plus catechol	31.8; 29.3	
Plus P.P.D. and catechol	95.6; 111.6	
Boiled carrots:		
Control	0.0	
Plus P.P.D	20.3	
Plus P.P.D. and catechol	73.5	
Plus P.P.D.; catechol, and M/1,000 HCN	10.3	
Dye controls:		
Catechol	0.0	
P.P.D	17.7	
P.P.D. and catechol	17.1	

\*P.P.D. = p-phenylene diamine; 10 mg. of P.P.D. or catechol where mentioned.

Table 6 shows that carrots which had been killed by boiling and gave no measurable oxygen consumption failed to catalyze the oxidation of p-phenylene diamine but did, however, give a marked cyanidesensitive catalysis of oxygen uptake in the presence of a mixture of p-phenylene diamine and catechol. (A slight temporary catalysis was observed in the presence of catechol alone.) A graph showing catalysis of the oxidation of this mixture by dead as well as by living roots is presented in figure 3. No control with p-phenylene diamine + catechol in the absence of carrots was run in the experiment graphed, but reference to table 6 shows that the rate of auto-oxidation is fairly low, less than 20 per cent of the rate with dead carrots as graphed in figure 3.

In the past, investigators of the qualitative nature of plant respiration have commonly depended on the use of color reactions with oxidizable artificial dye substances rather than on determinations of oxygen consumption. Apparently in some cases proper attention has not been paid to the importance of pH, the possibility of negative tests due to reduction as well as oxidation of the dye by the tissue, and the possibility of catalysis of dye oxidation by thermostable cell components not concerned in respiration.

Bhagvat and Richter (1938) report fairly general catalysis of oxidation of various phenolic compounds in the presence of small amounts of metallic ions; copper, iron, nickel, cobalt, and manganese. They have also demonstrated pseudophenolase activity—that is, they have isolated from the crab, Cancer Pagurus, a hemocyanin showing very definite phenolase activity, but still activity of a very much lower order of magnitude than that of true phenolases, such as Keilin and Mann's (1938) catechol oxidase. Our results with dye reactions of dead carrots are a further admonition to use care in the interpretation of this type of test.

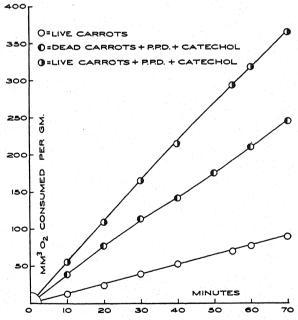


Fig. 3. Catalysis by dead carrots of the oxidation of p-phenylene diamine + catechol by molecular oxygen.

The shift in oxidase on growth of carrot leaves.—When CO, HCN, and NaN<sub>3</sub> were used as respiratory poisons on immature leaves (inner leaves of the rosette, 3 cm. or less in length), inhibition of respiration was found with all three poisons, as may be seen from table 7. However, the respiration of ma-

Table 7. Inhibition of the respiration of young carrot leaves by carbon monoxide, cyanide, and azide.

mm.3 0 <sub>2</sub> consumed	Percentage inhibition		
$95\% \ \mathrm{N}_2, 5\% \ \mathrm{0}_2$	95% CO, 5% 0 <sub>2</sub> (dark)		
381	230	40	
330	214	35	
Unpoisoned	Poisoned		
10-3 M c	yanide		
513	141	72	
418	140	67	
563	195	65	
585	230	61	
544	183	66	
10-3 M	azide		
601	262	56	
587	254	57	
575	255	56	
714	268	62	

ture leaves (outer leaves of the rosette, at least 25 cm. long) was not inhibited by any of these poisons, see table 8. The failure of these poisons to inhibit the respiration of mature leaves cannot be due to failure to penetrate the cells for the following reasons: (1) leaf tissue that is readily permeable to the gases CO<sub>2</sub> and O<sub>2</sub> can hardly be impermeable to the gas CO; (2) cyanide occasionally caused a respiratory stimulation; (3) no respiratory inhibition was found even when the leaves had been allowed to stand with their petioles in NaN<sub>3</sub> for 16 hours.

Table 8. Failure of carbon monoxide, cyanide, and axide to inhibit the respiration of mature leaves.

	mm. $^3$ $^02$ consumed/gm.				
Minutes	95% N <sub>2</sub> , 5% 0 <sub>2</sub>	95% CO, 5% 0 <sub>2</sub> (dark)			
40	278	256			
40	271	274			
40	318	355			
30	210	193			
30	262	248			
	Unpoisoned	Poisoned			
	10-3 М	cyanide			
30	210	212			
30	215	212			
60	455	576			
40	277	409			
40	286	433			
	10-2 M	eyanide			
50	339	394			
	10-3 N	I azide			
30	210	204			
30	177	194			
	10-2 N	I azide			
50	475	475			
50	411	423			

From these experiments we may conclude that a qualitative change in the respiratory mechanism occurs on maturation of the carrot leaves. It may be noted that on a wet weight basis, young carrot leaves respire more than twice as fast as mature leaves and almost ten times as fast as root tissue.

The fact that the normal respiratory rate of mature leaves was of the same order of magnitude as the residual rate of poisoned young leaves (see table

Table 9. Summary of respiratory rates with and without cyanide or azide for roots, mature leaves, and young leaves.

mm. $^3$ $^02/\mathrm{gm./hr.}$				
	Unpoisoned	10 <sup>-3</sup> M cyanide	10-3 M azide	
Root	91.2 (12)ª	21.9 (3)	22.5 (6)	
Mature leaf	439 (10)	537 (5)	398 (2)	
Young leaf	1,133 ( 9)	355 (5)	520 (4)	

<sup>&</sup>lt;sup>a</sup> The figures in parentheses indicate the number of determinations used in calculating the average.

9) suggested a speculation. It may be that two qualitatively different oxidase systems are operative in the young leaf, one of which, the cyanide-azide-CO sensitive one, is lost during development while the basic insensitive system persists throughout development. The simultaneous operation of two distinct oxidases has recently been very beautifully demonstrated by Stannard (1939); by use of sodium azide Stannard has separated the normal resting respiration of frog muscle from the extra respiration which is superimposed upon this normal respiration when the muscle is stimulated by caffeine or by electrically or chemically induced contracture.

Although catechol oxidase, an enzyme inactivated by cyanide, azide, and CO (Keilin and Mann, 1938), has long been considered a common plant oxidase, it is interesting to note that the respiration of the mature leaves of both higher plants recently investigated in this laboratory, wheat (Allen and Goddard, 1938) and carrots, has not been inhibited by these poisons. Further, the light reversal of the carbon monoxide inactivation of the primary oxidase of the carrot root shows that this respiration is not due to catechol oxidase.

### SUMMARY

The respiration of carrot root is reversibly inhibited by low concentrations of cyanide and azide. Cyanide at a concentration of  $10^{-2}$  M, however, does not completely inhibit the root respiration.

The respiration of carrot root is also inhibited by carbon monoxide; this inhibition may be reversed by light.

The enzyme which combines with carbon monoxide has an affinity for oxygen in the range of 9 to 13 times its affinity for carbon monoxide. This has been calculated according to Warburg's equation and our values agree well with Warburg's values for yeast.

These results suggest the operation of an enzyme similar to or identical with the cytochrome oxidase (indophenol oxidase) of yeast.

The light reversal of carbon monoxide inhibition of respiration indicates that catechol oxidase, long held to be the common respiratory enzyme of higher plants, is not the enzyme involved in carrot root respiration.

Carrot roots killed by boiling, though they do not consume oxygen, still give a positive Nadi reaction; they also catalyze (as determined by O<sub>2</sub> consumption) the oxidation of a mixture of p-phenylene-diamine and catechol.

The respiration of immature carrot leaves is similar to that of roots in being inhibited by NaN<sub>3</sub>, HCN, and CO, but when the leaves become mature, the respiration is not inhibited by any of these poisons.

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### THE SELENIFEROUS ASTRAGALUS OSTERHOUTII JONES 1

### O. A. Beath

CONSIDERABLE STUDY has been directed during the past few years to species in the Lonchocarpi group of Astragali as possible carriers of selenium. The reason given for anticipating this characteristic development was based upon the analogy of the Lonchocarpi and Bisulcati groups. According to Jones (1923) "This group (Lonchocarpi) is nearest related to the Bisulcati because of its compressed pod inclined to be bisulcate, one-celled, stipitate and opening only along the ventral suture but in other features it approaches the Homalobi." He further states: "The Lonchocarpi group doubtless has a relationship, though a loose one, with the racemosus part of the Galegiformes being a little more aberrant than A. drummondii. It is, like A. racemosus, a group of the alkaline plains."

The seleniferous character of all the species of the Bisulcati group and A. racemosus in the Galegiformes has been quite definitely established (Beath et al., 1939a, 1939b). In the Lonchocarpi group special consideration has been given to two valid species—viz., A. lonchocarpus Torr. and A. osterhoutii Jones. The former occurs (Jones, 1923) widely distributed from Pioche, Nevada, northward to Ferguson Spring near Ibapah, Utah, eastward from Pioche north of the Grand Canyon along the Virgin (north of Cedar City) and the Kanab region, throughout the Navajo Basin drainage eastward through Durango and the Little Colorado, to the Rio Grande, thence northward to Pueblo and Canyon City. A. osterhoutii has not been reported to occur outside of Grand County, Colorado.

Astragalus lonchocarpus has not been found to absorb selenium in toxic amounts. Representative samples have been obtained from Colorado, New Mexico, Utah, and Idaho. Field observations indicate that it is not necessarily confined to soils of a seleniferous character. A. lonchocarpus is regarded by stockmen to be a desirable range plant (Goodding, 1939). Consequently it seems quite probable that this species has no appreciable toxicity insofar as selenium involvement is concerned. On the other hand, A. osterhoutii is to be considered as a positive selenium carrier. Furthermore, its growth is associated exclusively with seleniferous shales. A representative collection was obtained July 3, 1939, six miles north of Kremmling, Colorado, on Niobrara shale. The plants at that time were in the early seed stage. The selenium value on air dried plants was found to be 2,678 parts per million. Later collections during July from the same general area north of Kremmling were submitted to selenium tests. All were definitely positive. The seleniferous character of A. osterhoutii has, therefore, been clearly established. It has also been observed that the plants, green or dry, emit the selenium odor so characteristic of many of the seleniferous Astragali (Beath et al., 1939a).

<sup>1</sup> Received for publication September 8, 1939.

In the same Kremmling area the author found other selenium-bearing Astragali, notably A. haydenianus Gray and A. pattersonii Gray. It was interesting to observe that for comparable stages of growth the A. osterhoutii was much more seleniferous than A. haydenianus or A. pattersonii under identical soil conditions.

A large collection of A. osterhoutii was made during July for animal feeding tests. A pound of air dry material (whole plant) was sufficient to produce death in a sheep within a few hours. So far as the author is aware no previous mention has been made of the toxic character of this species of Astragalus.

Beath et al. (1939a, 1939b) and Trelease (1939) have presented evidence that indicates a tendency for only certain species of Astragalus to be natural absorbers of selenium. Beath et al. (1939a, 1939b) have pointed out that exceptions occur. One of the most prominent cases involves the non-seleniferous A. drummondii Dougl. and the positive seleniferous A. racemosus Pursh. Both are classified by Jones (1923) as occurring in the Galegiformes group. No reason can be given at this time as to why this diversity of selenium absorption occurs in the Galegiformes group.

The significant point brought out in the chemical study of two valid species in the Lonchocarpi group is that one is seleniferous and the other is not. The author has not been able to obtain specimens of the other species in the Lonchocarpi group—viz., A. kaibensis Jones and A. duchesnensis Jones.

From a genetic point of view one may well raise the question as to why A. osterhoutii is consistently seleniferous and A. lonchocarpus is non-seleniferous. Is it because of a carry-over of the bisulcate influence (selenium absorption) into the A. osterhoutii that was left out of A. lonchocarpus? If not the bisulcate influence, could it be assigned to an A. racemosus trend? Or is it a dual influence? In any event it would seem to the author that this interesting diversity offers a geneticist an opportunity to investigate a problem unique in its field. If the seleniferous Astragali were scattered here and there throughout the western United States independent of any group association, it would be clearly impossible to assign any genetic basis for their selenium absorbing properties. Since preliminary data strongly indicate the possibility of a segregation, in a majority of cases, of those species that are seleniferous into definite groups, it would appear logical to conclude that a basic reason exists for such a behavior.

### SUMMARY

Astragalus osterhoutii and A. lonchocarpus are valid species belonging to the Lonchocarpi group. A. osterhoutii is confined in its growth to seleniferous soils and in addition is capable of absorbing selenium in toxic quantities. Consequently it may be

looked upon as a livestock hazard and also as a soil contaminator.

A. lonchocarpus is not confined in its growth to a seleniferous soil. Samples from a diversity of soils from four western states have been analyzed for selenium with negative or very low results. It is concluded that this species does not absorb selenium in toxic amounts even from soils known to contain appreciable amounts of soil selenium. It is regarded by stockmen to be a safe forage.

The analogy in some respects of species in the Lonchocarpi group to those in the Bisulcati and Galegiformes would suggest that a selenium requirement has been retained through the ontogeny of A. osterhoutii that is found to be lacking in A. lonchocarpus.

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# CHROMOSOME NUMBERS OF CALIFORNIAN SALVIAS 1

### Wm. S. Stewart

TAXONOMIC DIFFICULTIES with the 18 Californian species of Salvia suggested that a determination of their chromosome number might be useful. The results of such determinations are reported in this

In the New World, the genus Salvia is represented chiefly by the subgenus Calosphace (Epling, 1939). This is a complex of nearly five hundred species, which are nevertheless notably uniform in floral and vegetative habit for so large a group. Within this complex approximately ninety sections or species groups have been recognized. The species which comprise each of these groups are in general similar in growth form and floral habit. This subgenus is predominantly Andean and Mexican, reaching as far west in the United States as Arizona.

In the state of California, however, is centered a group of species of Salvia, eighteen in number, which have been variously treated by systematic writers. Five of these species, S. Columbariae, Carduacea, Greatae, californica, and funerea, have always been referred to Salvia. The remainder have been considered by some authors as generically distinct and in the past have been recognized as the genus Audibertia. Of these species, one, S. apiana, has even been proposed as the type of a third genus, Ramona. The present position of writers on the Californian flora is to refer all these species to Salvia. This position has also been adopted by the most recent monographer who has referred the whole number to Audibertia as a section of Salvia.

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The author wishes to express his thanks and appreciation to Dr. Carl C. Epling of the University of California at Los Angeles for aid in collecting the material and for advice on the taxonomic discussion and to Dr. Th. Dobzhansky of the California Institute of Technology for consultation in making the chromosome counts.

As might be expected, this diverse taxonomic treatment reflects the fact that, unlike the species groups of Calosphace, the species groups of Audibertia are quite diverse morphologically, both with respect to growth form and floral habit. However, from a morphological standpoint alone there are valid reasons for recognizing these groups as all being similar and hence better regarded as species which are peripheral segregates of Salvia (Epling, 1938a). Moreover, certain observations suggest that the species of Audibertia may hybridize in nature when occupying the same territory. The frequency and nature of these hybrids, which appear to be sterile, as well as the nature of the variation between the species has suggested the possibility that this group as at present constituted has had its origin, at least in part, through allopolyploidy (Epling, 1938b). Eleven presumed hybrids among different Californian species of Salvia have been recorded by taxonomists (Jepson, 1923; Munz, 1935; Epling, 1938a).

Chromosome counts were made in pollen mother cells at diakinesis stage as well as in metaphase plates in transverse sections at root tips. The anthers for the pollen mother cell determinations were collected in the field and preserved either in formalin-acetic-alcohol or in a solution consisting of three parts 95 per cent alcohol and one part glacial acetic acid. No difference in effect of the two fixatives was observed.

The root tip material was obtained using transplants from the native environment to a sand culture medium. They were watered once a week with Hoagland's nutrient solution to which had been added vitamin  $B_1$ , a root growth hormone. This was added since it has been shown by Addicott (1939) that vitamin  $B_1$  is essential for meristem cell divi-

sion in roots. For the purposes of this investigation this was of course desirable. The main root stalk had generally become well covered with young rootlets about three to four weeks after the transplantation. These were removed and fixed in Navaschin's solution for further study.

The root tip sections were cut 10 micra thick and stained with Heidenhain's iron-alum-haematoxylin. The pollen mother cell smears were made according to the method of McClintock (1929), using iron-aceto-carmine.

The results of counts are summarized in table 1. Here the species are arranged according to species groups or subsections as given by Epling (1938a). The species within a subsection form natural morphological units. Camera lucida drawings of representative metaphase plates from root tip preparations are shown in figure 1. The data show that there is no clear basic number of chromosomes for species of the section Audibertia; species with haploid numbers of 8, 11, 12, 13, and 16 are encountered. In other sections of the genus Salvia a variety of chromosome numbers is likewise established. Thus Yakovleva (1933) recorded species with n = 6, 7, 8, 9, 10, 11, 12, 16, 18, and 32. Moringa, Fukushima, and Yamasaki (1929) also report n = 8 for S. nipponica var. argutidens. It thus seems impossible to speak of a single basic chromosome number either for Salvia as a whole or for the section Audibertia alone. Regarding the chromosome number for the family Labiatae, Wanscher (1934) observes that the number 8 has been found in five out of nine genera and the numbers 9 and 12 in two genera each. He concludes that probably in this family there has been development from a 4 system.

In the case of S. carduacea the haploid number of chromosomes in several pollen mother cell counts was determined as 12, whereas both Scheel (1931) and Yakovleva (1933) indicate it to be 16. This discrepancy may be due to a racial difference in the materials used. Likewise in the case of S. Columbariae none of the published chromosome numbers agree. The same explanation given for S. Carduacea may hold in this case also. In this regard it is significant that both these species have wide distributions, since they extend over a territory about a thousand miles in length. This would afford an opportunity for the formation of genetically different races. It should be remarked, however, that both species are unusually stable and present no evidence of morphological differentiation. S. Columbariae and S. Carduacea are the only annuals in the section Audibertia, and they show low chromosome numbers, n being equal to 8 and 12, respectively. Presence of lower chromosome numbers in annuals and higher numbers in perennial species of the same ge-

Table 1. Chromosome numbers in the California Salvias.

Chromosome numbers					
	Our data		Other authors		
Subsections and species	n	2n	n	2n	
Echinosphace					
Carduacea	12	••	16	32	(Scheel, 1931. Yakovleva, 1933.)
funerea	••	• •	••	•	
Greatae	•	•	••		
Pycnosphace				•	
Columbariae	8	••	16	26	(Carlson & Stuart, 1936, Yakov- leva, 1933.)
Greeneostachys					
spathacea		26			
Parishiella					
Brandegei	• •				(Carlson & Stuart, 1936.)
mellifera	16	• •	15	•••	
Munzii	••	24			
Jepsonia					
sonomensis	• •	32			
leucophylla	12	•	•••		
subsp. argentea					
subsp. Gilmani		22			
subsp. Mearnsii					
subsp. pilosa		32			
subsp. typica					
pachyphylla					
Vaseyi					
apiana	16	32	15		
Clevelandii		32		• •	(Carlson & Stuart, 1936.)
mohavensis					
eremostachya		24			

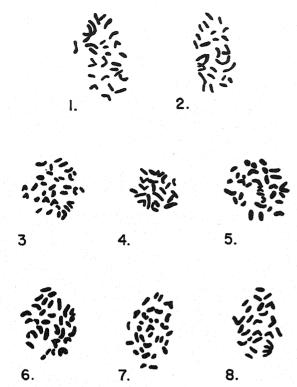


Fig. 1. Camera lucida drawings of metaphase plates from transverse root tip sections of: 1, Salvia spathacea, 2n = 26; 2, S. Munzii, 2n = 24; 3, S. sonomensis, 2n = 32; 4, S. carnosa subsp. Gilmani, 2n = 22; 5, S. carnosa subsp. pilosa, 2n = 32; 6, S. apiana, 2n = 32; 7, S. Clevelandii, 2n = 32; 8, S. eremostachya, 2n = 24. Magnification  $1800 \times$ , except No. 4 which is  $1200 \times$ .

nus is indeed not a rare phenomenon (Müntzing, 1936).

It is interesting to note that S. spathacea is one of the most singular species of the Salvias in California and that it is also unique in its chromosome number (2n = 26).

Another point of some interest is the diploid chromosome number found in S. carnosa subsp. Gilmani. This subspecies is largely restricted to the Panamint Mountains, west of Death Valley. It was found to have a diploid compliment of 22 chromosomes in contrast to 32 chromosomes found in S. carnosa subsp. pilosa which occurs throughout the eastern part of California. From a genetic point of view this would indicate that these two "subspecies" have reached the species rank, although they are morphologically similar. Other subspecies of this species were not investigated.

It should be pointed out that the chromosome numbers reported in this paper were established from material collected at only one locality for each species. Hence, this does not preclude the possibility that other "races" of the same species may give different results. Most of the species studied have a wide distribution.

### SUMMARY

A determination of chromosome numbers in species of Californian Salvias showed that: Paralleling the morphological diversity of the group, diverse haploid chromosome numbers of 8, 11, 12, 13, and 16 are found. Within the species S. carnosa the subspecies Gilmani has a haploid number of 11, whereas the subspecies pilosa has a haploid number of 16. There was neither evidence for nor against the suggestion that the species could have arisen either as the result of hybridization alone or as allopolyploids.

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# RESPONSE OF TOMATO PLANTS TO $\beta$ -NAPHTHOXYACETIC ACID <sup>1</sup>

S. C. Bausor

In a previous paper  $\beta$ -naphthoxyacetic acid was reported as a growth-substance, stimulating growth or inhibiting it, according to the concentration used, as indicated by curvatures resulting from unilateral treatment, and later inducing the formation of root-primordia (Bausor, 1939). The present paper is a further analysis of the activity of  $\beta$ -naphthoxyacetic acid.

Methods and materials.—Potted Marglobe tomato plants about eight inches high were used as experimental plants. These were treated with lanolin solutions of  $\beta$ -naphthoxyacetic acid in concentrations of 1.0, 0.1, 0.01, and 0.001 per cent. Anhydrous lanolin, employed as the solvent for the experimental material, was used pure as a control.

The 1.0 per cent solution of  $\beta$ -naphthoxyacetic acid in lanolin was made by weighing both substances (100 mg./10 g.), while the other concentrations were made by dilution (1 g. of 1 per cent plus lanolin to make 10 g. = 0.1 per cent; 1 g. of 0.1 per cent to make 10 g. = 0.01 per cent; and 1 g. of 0.01 per cent to make 10 g. = 0.001 per cent). The material was applied unilaterally, and resulting curvatures were indicated as negative when the organ bent away from the paste or positive when it bent toward the paste, as is the convention. Seventy-four plants were used in all, eight of which served as controls.

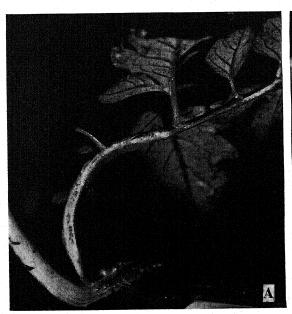
Experimental results.—Range of activity.—In an experiment designed to test the relative activity of various dilutions of  $\beta$ -naphthoxyacetic acid, four

1 Received for publication July 12, 1939.

groups of eight plants each were used, four of the plants in a group being treated on one side of the stem, the other four on the lower side of the petiole. The groups were treated, respectively, with 1.0, 0.1, 0.01, and 0.001 per cent dilutions. A fifth group of four plants served as controls and was treated with pure lanolin.

It was found that concentrations ranging from 1.0 to 0.01 per cent were physiologically effective, while a 0.001 per cent dilution was inactive. This was true whether the lanolin pastes were applied unilaterally at the apex of a stem, several internodes removed from the apex, or to the lower or upper sides of a petiole. (In a previous experiment various concentrations were applied to the upper side of the petiole, but since the lanolin gravitated to the lower side after a day or so, this method was discontinued.)

Rooting response.—About ten days after treatment an abundance of root primordia were in evidence on those plants whose stems or petioles were treated with 1.0 per cent  $\beta$ -naphthoxyacetic acid (fig. 1). Roots appeared not only on the stems or petioles where the material was smeared, but also below it, extending to the base of the stem. When the paste was applied at the apex, growth in height ceased, and eventually root primordia developed to the very apex of the plant (fig. 2). Stems treated with a 0.1 per cent paste also produced root primordia but not so abundantly as with 1.0 per cent, and only where the paste was applied to the stem. However, the plants on which petioles were treated with



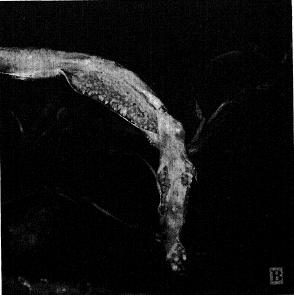


Fig. 1. Root primordia developing on stems and petioles of tomato plants after application of a 1.0 per cent lanolin solution of  $\beta$ -naphthoxyacetic acid. A. Root primordia bursting through upper side of a slightly twisted petiole. Lanolin paste on lower side is visible. B. Base of stem of plant which was treated at the apex, showing profuse and crowded root primordia breaking through the epidermis.

0.1 per cent did not develop root primordia, nor did any of the plants subjected to 0.01 per cent.

Bending responses.—Stems.—Curvatures produced by the different concentrations were rather interesting. With a 1.0 per cent paste applied to the stem, either at the apex or several internodes below, the first sign of activity in most cases was in the region of the stem below the point treated with the material. In a few cases the resulting curvature was at right angles to the paste and difficult to interpret, but in most cases it was definitely negative. This is indicative of a stimulating effect, assuming that the

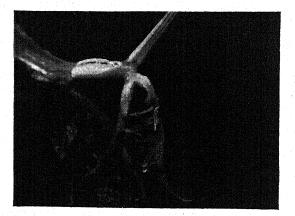


Fig. 2. Apex of tomato plant treated with 1.0 per cent  $\beta$ -naphthoxyacetic acid, showing white swollen areas of root primordia which have not yet emerged. Root primordia are developing right up to the apical meristem.

material diffused downward on the same side as that to which the paste was applied. Later the region contacted by the paste produced a strong positive curvature. The combination of the two effects resulted in a sigmoid curve. The negative curvature was so intense that the habit of the plants was changed from erect to prostrate, and when the paste was applied to the apex, the cessation of growth there made the effect permanent, except for the development of axillary buds in the lower nodes.

With a 0.1 per cent paste the bending of the stem was at first strongly negative at the place of contact with the material, sometimes followed by a slight negative curvature below. The region in direct contact subsequently developed a strong positive curvature. A 0.1 per cent paste applied to the apex likewise caused a cessation of growth and a prostrate habit, but more axillary branches developed than on plants similarly treated with 1.0 per cent  $\beta$ -naphthoxyacetic acid. With 0.01 per cent the reaction occurred only in the area treated, producing a strong negative curvature. When applied to the apex it did not stop the growth of the plant. A 0.001 per cent paste produced no curvatures.

Leaves.—A 1.0 per cent paste on the lower side of a petiole situated either at the apex of the stem or below, produced a strong negative curvature in the internode below and a strong positive curvature in the petiole. The latter was not the typical epinas-

tic response, but a more extensive growth, the petiole curving strongly wherever the paste touched it.

Petioles similarly treated with a 0.1 per cent solution curved upward at first, and the internode below it also curved negatively. The following day the curvature of the petioles was positive, but at the termination of the experiment (10 days) it was again negative.

With 0.01 per cent the petioles bowed upward negatively, but the reaction became less conspicuous as time went on, and at the close of the experiment they had resumed almost normal position. Only a slight twist indicated their previous induced excursion. The 0.001 per cent solution had no effect upon the petiole.

Rate of response.—The reaction is very rapid. In most cases a noticeable curvature, following unilateral treatment, occurs within one hour, and it becomes fairly pronounced at the end of the second hour (fig. 3, a-d).

Of the thirty-two plants experimented upon for reaction time, all but three responded within 80 minutes or less. These three reacted after two hours. In the others, curvatures were fairly pronounced at the end of two hours (fig. 3c). In a typical plant the reaction to 1.0 per cent  $\beta$ -naphthoxyacetic acid was as follows: 9:36 a.m., treated the second internode from apex; 10:40 a.m., slight negative curvature below treated area; 10:58 a.m., negative curvature greater; 11:45 a.m., very strong negative curvature; 2:57 p.m., definite positive curvature at place of contact, and below it, as before, a negative curvature; 6:18 p.m., about the same; 9:50 p.m., very strong positive curvature by tissues in contact with the paste and a very strong negative curvature below.

Although the response was somewhat different with 0.1 per cent, the time of reaction was comparable. A typical experiment follows: 9:18 a.m., treated apical internode; 10:27 a.m., slight negative curvature where paste is applied; 11:55 a.m., about the same; 1:56 p.m., strong negative curvature at place of contact, slight negative curvature below; 5:05 p.m., slight positive curvature at place of contact, strong negative curvature below.

The onset of these reactions is similar to that reported by Zimmerman and Hitchcock (1936). They noted that the response of the tomato to lanolin preparations of indolebutyric acid, indoleacetic acid, naphthaleneacetic acid, etc., occurred within one hour and that the maximum bending occurred within six to ten hours.

Discussion.—The peculiar reaction of tomato plants to unilateral treatment with 1.0 or 0.1 per cent solutions of  $\beta$ -naphthoxyacetic acid in lanolin, producing both a stimulating and an inhibiting effect, may be reasonably explained by assuming that the material becomes diluted as it diffuses into the plant and downward. This dilution probably approaches 0.01 per cent, since at this concentration a definite negative curvature is produced, and it must be greater than 0.001 per cent, at which no reaction occurs. This would account for the negative curvatures which take place below the regions smeared

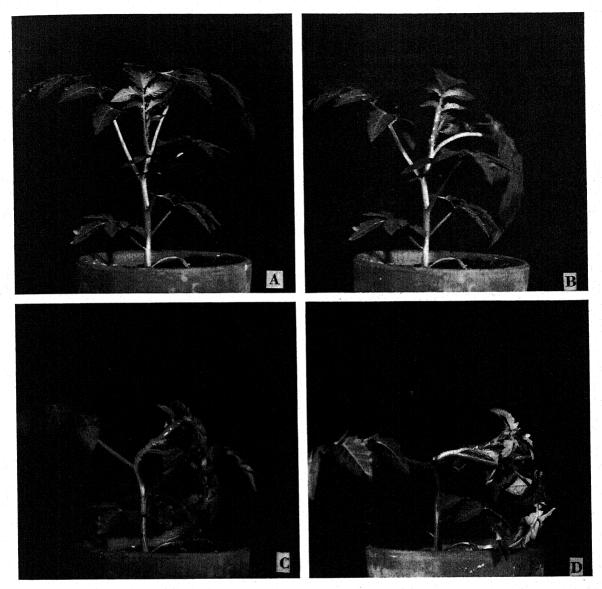


Fig. 3. Bending response and reaction time. A. Tomato plant before treatment. B. 54 minutes after a 1.0 per cent lanolin solution of  $\beta$ -naphthoxyacetic acid was applied to the lower side of the apical petiole, showing negative curvature below paste (11:01 p.m.). C. 106 minutes after treatment. Strong negative curvature below paste; slight positive curvature at region contacted by paste not obvious in photograph (11:53 p.m.). D. 154 minutes after treatment. A very strong negative curvature of stem and epinasty of leaf is in evidence below point of application, and a slight positive curvature in the treated petiole (12:41 a.m.).

with a 1.0 per cent paste, and for the negative curvatures occurring at first where the 0.1 per cent paste was applied, later followed in the same place by a reversal to a positive curvature. It seems that the  $\beta$ -naphthoxyacetic acid becomes dilute enough as it diffuses into the contacted area to produce a stimulation to the growth processes. But as more of the growth-substance enters the tissues, the original negative curvature disappears and a strong positive curvature is found in its place, indicating that growth has been inhibited on the treated side and is now slower than that of the opposite, untreated side.

The inhibition of growth in length by concentrations of 0.1 and 1.0 per cent further bears out this contention.

Zimmerman and Hitchcock (1936) reported that low concentrations of growth-substances in lanolin induced negative bending of stems and leaves, while high concentrations induced positive bending. For naphthaleneacetic acid, which is structurally similar to  $\beta$ -naphthoxyacetic acid, they found that concentrations ranging from 0.002 per cent to 1.0 per cent produced negative curvatures, and percentages of 2 or more caused positive bending.

The negative curvature which occurs at first with 1.0 and 0.1 per cent  $\beta$ -naphthoxyacetic acid places the stem out of line of the direction of the gravitational pull, but the magnitude of the positive curvature which follows indicates that the reaction is not solely, if at all, a secondary, negative, geotropic response to lateral displacement.

With a 0.01 per cent concentration, however, the negative curvature almost disappears (if the treatment is at the apex) as a result of subsequent faster growth on the lower side of the horizontally curved stem responding to a geotropic stimulus. When applied below the tip on slower growing tissues, the negative curvature does persist as a crook in the stem, the geotropic curvature occurring above it. Since it is now known that geotropic responses are influenced or caused by natural plant hormones and growth substances (Boysen Jensen, 1936; Zimmerman and Hitchcock, 1936, 1938), this secondary effect probably is due to the radial distribution of natural hormones overcoming the effect of the stimulus of the artificial growth substance.

The subsequent development of root primordia only with concentrations which inhibit growth in length probably indicates a correlation between the two processes. The presence of root primordia up to the apical meristem seems to indicate a direct formative effect and not a secondary result of the cessation of growth, since the root primordia are initiated in tissues at the apex, which are developmentally

immature.

### SUMMARY

 $\beta$ -naphthoxyacetic acid provokes a growth response in intact tomato plants in concentrations ranging from 1.0 to 0.01 per cent, while a 0.001 per cent solution in lanolin is without effect.

A 1.0 per cent paste causes a negative curvature below the point of application, be this on the stem or petiole, and a positive curvature in the treated region. A 0.1 per cent paste causes a negative curvature below the point of application and at first also where the paste contacts the tissues. The latter subsequently becomes positive. A 0.01 per cent paste is effective only in the treated area, causing a negative curvature.

The above reactions with different concentrations of  $\beta$ -naphthoxyacetic acid is explained on the assumption of dilution of the substance as it diffuses from the lanolin into the plant and from cell to cell,

The development of root primordia follows the application of 1.0 and 0.1 per cent pastes, both of which also inhibit the growth of treated tissues.

The rate of reaction is very rapid. The first noticeable effect occurs usually within one hour, and a very conspicuous reaction occurs after two hours.

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### CYTOLOGY OF THE GENUS LESPEDEZA<sup>1</sup>

### W. P. Pierce

THE GENUS Lespedeza Michaux, according to Pieters (1934), includes about 124 species, of which 17 are indigenous to the United States. The majority are native to eastern Asia, particularly China, Japan, Siberia, and Manchuria. A few species have been reported from Australia, Java, and Mauritius. A member of the Leguminosae, the genus is placed by Taubert (1894) in the subfamily Papilionatae, tribe Hedysareae, subtribe Desmodiinae.

Cytological study of the subtribe Desmodiinae has been meager. Of 15 genera, only Lespedeza and Desmodium Desv. have been reported on to date, and the reports concern chiefly chromosome num-

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bers. In Desmodium the haploid number 11 has been found in four species: D. perpesium (Kawakami, 1930), D. grandiflorum (Cooper, 1936), D. canadense, and D. tortuosum (Senn, 1938).

In Lespedeza, Kawakami (1930) reported the haploid number as 9 in L. bicolor, L. cyrtobotrya, L. homoloba, L. Sieboldi, and L. Sieboldi var. albiflora. Cooper (1936) reported the diploid number as 18 in L. variegata and L. sericea, but 20 in L. tomentosa and L. stipulacea. In L. daurica he found the diploid number to be 36. For each of the species studied Cooper presents a short description of chromosome morphology and range of length. One pair of satellite chromosomes is present in L. variegata and L. tomentosa. Cooper suggests, in view of the numbers thus far determined in the genus, that nine is probably the basic number.

Senn (1938) attempts to relate chromosome number in the Leguminosae with taxonomic characters and with the herbaceous or woody habit; he dis-

cusses also the relation of polyploidy to geographic distribution. Although admitting the insufficiency of present information, he suggests that chromosome numbers in Lespedeza and Desmodium may aid in distinguishing these genera, the basic number for Lespedeza appearing to be 9 and that for Desmodium 11. He suggests that further investigation may show that in Amorpha and Lespedeza, the distribution of polyploids is conspicuously more northern than that of diploids. Yet he points out that such evidence is inconclusive, inasmuch as in ten genera, including Lespedeza, diploid species have a wider distribution than polyploids.

MATERIALS AND METHODS.—The plants used in the present study were grown from seeds obtained mostly from the U. S. Department of Agriculture, through the kindness of Dr. A. J. Pieters and Mr. Roland McKee of the Division of Forage Crops and Diseases. Some seeds were taken from specimens in the herbarium of the University of Wisconsin, and a few were collected from plants growing in the field.

To secure a reasonable percentage of germination, it was found necessary in almost all cases to scarify the seeds. Exposure to a temperature below freezing, as well as to intermittent sunlight, aided in the germination of the rather old seeds of a few species. Determinations of chromosome numbers were made in most cases from the primary roots of seedlings allowed to grow for a period of a week to ten days in sand, then fixed in Belling's, Karpechenko's, or Randolph's modification of Navashin's

TABLE 1.

Species	Collection number	Haploid	Diploid	Author
ect. I. Archilespedeza Taub.				
A. Macrolespedeza Maxim.				
L. bicolor Turcz		9		Kawakami, 1930
L. bicolor Turcz	81644		22	Pierce
L. cyrtobotrya Mig		9		Kawakami, 1930
L. cyrtobotrya Miq	F. P. I. 82092		22	Pierce
L. cyrtobotrya var. pedunculata Nakai	F. P. I. 104066		22	Pierce
B. Eulespedeza Maxim.				
L. violacea Pers			20	Pierce
L. Stuvei Nutt			20	Pierce
L. floribunda Bge	90996		22	Pierce
L. virgata DC	F. P. I. 90167		22	Pierce
L. repens Bart	F. C. 21051		20	Pierce
			18	
L. sericea Benth	•••••			Cooper, 1936
L. sericea Benth	•••••	9	• •	Pierce
L. capitata Michx			20	Pierce
L. variegata Cambess	F. C. 21866	••	18	Cooper, 1936
L. variegata Cambess	F. C. 21866	• •	20	Pierce
L. hirta (L.) Hornem. (=L. villosa Pers.)	21069	• •	20	Pierce
L. pilosa Maxim	F. C. 12086		20	Pierce
L. tomentosa Siebold (= L. villosa Pers.)	••••	••	20	Cooper, 1936
ct. II. Campylotropsis Bge.				
L. macrocarpa Bge	F. C. 93998		22	Pierce
ct. III. Microlespedeza Maxim.				
L. striata var. Kobe	F. C. 22242		22	Pierce
L. stipulacea Maxim. (= L. striata Hook. et Arn.?)			20	Cooper, 1936
	•••••	••	20	Cooper, 1936
ction undetermined.	<b>77 6 6</b> 1.116			
L. procumbens Michx	F. C. 85228	• •	20	Pierce
L. frutescens (L.) Britton		• •	20	Pierce
L. latissima Nakai	F. C. 19283	• •	20	Pierce
L. inschanica (Maxim.) Schind	F. P. I. 88315	• •	20	Pierce
L. Maximowiczii Gandog. (= L. bicolor Turcz.)	82482		22	Pierce
L. Thunbergii Nakai	F. P. I. 25009		22	Pierce
L. virginica (L.) Britton (= L. sessiliflora Michx.)			22	Pierce
L. japonica var. intermedia (Nakai) Nakai	F. P. I. 82094		22	Pierce
L. robusta Nakai	F. P. I. 108229		22	Pierce
L. homoloba Nakai		9		Kawakami, 1930
L. Sieboldi Miq		9		Kawakami, 193
L. Sieboldi var. albiflora Schneid		9		Kawakami, 193
L. daurica Schind	F. C. 89740		36	Cooper, 1936
L. daurica Schind.	F. C. 89740	* *	ca. 44	Pierce
L. daurica var. Shimadai (Masamune) Masam. and	r. C. 65140	* * * * * * * * * * * * * * * * * * * *	ca. Tr	T TOLOG
	T D T noore		00 44	Diores
Hosokawa	F. P. I. 90353	• •	ca. 44	Pierce



Fig. 1-20.—All figures are of somatic equatorial-plate chromosomes from cells of root tips fixed in one of the Navashin fixatives, with the exception of fig. 10, which is of a dividing nucleus in a microspore, fixed in Carnoy's alcohol-acetic solution. All were drawn with the aid of a camera lucida. The magnification in all cases, ca.  $3000 \times -$  Fig. 1. L. bicolor. From a cell of the periblem (2n=22).—Fig. 2. L. bicolor. From a cell of the dermatogen.—Fig. 3. L. cyrtobotrya (2n=22).—Fig. 4. L. cyrtobotrya var. pedunculata (2n=22).—Fig. 5. L. violacea (2n=20).—

fluid, or in Flemming's medium solution. All gave fairly satisfactory results; perhaps the best were obtained with Randolph's formula.

Fixations of floral buds were made from plants growing in the field and in the greenhouse. Most species of Lespedeza produce both chasmogamous and cleistogamous flowers in the same inflorescence, usually the latter type only being fertile. The small size of the buds, and the presence of closely adherent hairy bracts, made fixation difficult. A variety of fixatives were tried, in some cases after pre-fixing in Carnoy's alcohol-acetic solution. To aid in penetration an aspirator also was used for short periods of time. It was finally apparent that satisfactory results could be secured only by partial dissection in a fixative under a binocular microscope.

Root tips were dehydrated with chloroform and imbedded in the usual manner. This method was found to harden buds to such an extent as to make sectioning very difficult. Dehydration with the aid of an anilin-cedarwood oil mixture proved more satisfactory since it avoids the use of the higher concentrations of alcohol. Sections were cut at a thickness of 8 to 10 microns. Smith's (1934) picric-acid modification of Newton's crystal violet-iodine was the stain most used for root-tip material. For floral buds the best results were obtained with Heidenhain's iron-alum haematoxylin.

The use of Belling's aceto-carmine method in the study of meiotic stages proved unsatisfactory, probably because of the extremely small size of the anthers

Observations.—Table 1 lists the species studied, seed collection number if the seed was acquired from the Department of Agriculture, chromosome number, and the author and date of each report of chromosome number. The species are arranged as far as possible to agree with Taubert's subdivision of the genus. Numbers listed under the present author's name are reported in this paper. Synonyms in parentheses are those retained in Index Kewensis.

Section Archilespedeza Taub., subsection Macrolespedeza Maxim.—L. bicolor Turcz. (fig. 1, 2).—The somatic (2n) chromosome number in this species, determined from division figures in the root tip, is 22. The chromosomes are well separated and easily counted. As appears from table 1, this number does not agree with that (n=9) reported for this species by Kawakami. For this reason the count here given was decided upon only after a considerable number of favorable equatorial plates were examined and drawn.

The chromosomes in this, as in most species of the genus, are small, varying in a typical complement from 1.5 to 3.0  $\mu$ . These measurements are presented with the realization that many factors, such

as the relative position of the cell in the root tip and the exact stage of contraction of the chromosome, may cause a considerable variation in chromosome length and width from cell to cell. For example, in figure 1, drawn from a cell of the periplem, the chromosomes appear somewhat longer and thicker than those in figure 2, from a cell of the dermatogen. Therefore the figures given denoting ranges of chromosome lengths may be considered useful in a relative sense only.

The chromosomes appear to fall into three general types (fig. 1a) according to the location of primary constrictions, the types not being sharply delimited. In some equatorial plates the constrictions are readily apparent; in others they are inconspicuous. Observations of the former type of figure would tend to indicate that in many cases primary constrictions are located at the region of a major bend in the chromosome. Secondary constrictions, although sometimes visible, were not recognizable in a sufficient proportion of cases to warrant discussion.

In the three main types of chromosomes referred to, the primary constrictions are, respectively, (A) median, (B) submedian, and (C) subterminal. In addition, chromosomes bearing satellites, designated as type (D), are often to be observed. Although lengths do not show very great differences, the chromosomes may further be distinguished as long, medium, and short.

In L. bicolor there appear to be at least three pairs of chromosomes of type A (median constriction), two long and one short. In this species most of the homologous pairs can be identified (fig. 1a). Type B (submedian constriction) is represented here by at least 3 pairs, all apparently of medium length. Two pairs of type C chromosomes (subterminal constriction) appear to be long, one pair very short, and one pair of medium length. A single short chromosome with an attached satellite was observed in several equatorial plates (fig. 2), but the homologous chromosome could not be identified. The approximate formula for a somatic complement (fig. 1a) may be represented: 2(3A + 3B + 4C + D).

L. cyrtobotrya Miq. 2n = 22 (fig. 3).—The chromosomes are similar in size and morphology to those of L. bicolor. The four types of chromosomes are again recognizable in the proportions: 2 (4A + 3B + 3C + D). Of type A two pairs are of medium length and two are short. All the type B chromosomes seem to be long or of medium length. It should be pointed out that it is difficult to distinguish with certainty, here as in other species, between chromosomes with median and those with submedian constrictions. The constrictions characteristic of type C are not very evident, but the bending of the chromosomes would indicate the presence of one long

Fig. 6. L. Stuvei (2n=20).—Fig. 7. L. floribunda (2n=22).—Fig. 8. L. virgata (2n=22).—Fig. 9. L. repens (2n=20).—Fig. 10. L. sericea, from a microspore (1n=9).—Fig. 11. L. capitata (2n=20).—Fig. 12. L. variegata (2n=20).—Fig. 13. L. hirta (2n=20).—Fig. 14. L. pilosa (2n=20).—Fig. 15. L. macrocarpa (2n=22).—Fig. 16. L. striata Kobe (2n=22).—Fig. 17. L. procumbens (2n=20).—Fig. 18. L. frutescens (2n=20).—Fig. 19. L. latissima (2n=20).—Fig. 20. L. inschanica (2n=20).

pair, one of medium length, and one short. As in L. bicolor, one pair of type D chromosomes (d, d, fig. 3) is present. Here they appear distinctive because of the large size of the satellites, and of the fact that these D-type chromosomes are nearly as long as the longest in the complement. In this species also Kawakami has reported the haploid number 9. The somatic number here reported was based upon the examination of a considerable number of favorable figures.

L. cyrtobotrya var. pedunculata Nakai 2n=22 (fig. 4).—As would be expected, this variety corresponds fairly closely in chromosome size and form with the typical L. cyrtobotrya. The chromosome formula is 2(4A+3B+3C+D). The relative sizes of the chromosomes of type A and C are much the same as those in the typical form. The chromosomes of one pair of type B seem shorter in the variety. The greatest contrast was observed in chromosomes of type D, which are of only medium length and bear comparatively small satellites.

Subsection Eulespedeza Maxim.—L. violacea Pers. 2n = 20 (fig. 5).—Seeds were secured from a herbarium specimen collected in Grant County, Wisconsin, in 1934. The seeds germinated poorly, probably because of their age, and satisfactory division stages were few. The figure shows that the chromosome morphology of this species is not very distinctive. As nearly as can be determined, it has the 2 (3A + 4B + 3C). The chromosomes of type A consist of one long pair and two of medium length; those of type B of one short pair and three of medium length; those of type C of two long pairs and one pair of medium length. No constrictions were visible in a number of these chromosomes. In the equatorial plates examined, no satellite chromosomes were observed. Chromosome lengths range from 1.3 to 2.3 μ.

L. Stuvei Nutt. 2n=20 (fig. 6).—This species also is native to the United States. The count was made from six equatorial-plate stages, most of which showed unusually clear chromosome morphology. The chromosome formula is 2(4A+4B+2C+D). Of the chromosomes of type A, three pairs are long and one is short. Type B includes one long pair and two of intermediate length. Both pairs of type C appear to be fairly long. A single pair of short chromosomes bearing very small satellites was observed in three cases. Chromosome lengths range from 1.6 to  $2.5 \mu$ .

L. floribunda Bunge. 2n=22 (fig. 7).—In this Chinese species not only is the number the same in L. bicolor and L. cyrtobotrya, but there is apparent also a fairly close resemblance in the chromosome complements, as shown by the formula: 2(4A+3B+3C+D). Of type A there are one long pair, one of medium length, and two short. All three pairs of type B are of medium length. Those of type C are all long. The two type D chromosomes are of medium length, with no evident constrictions, and bear minute satellites. Chromosome lengths range from 1.5 to  $2.5 \mu$ .

L. virgata DC. 2n = 22 (fig. 8).—The somatic complement of this Asiatic species appears distinctive because more than half of the chromosomes are less than  $1.6 \mu$  in length. The range is from 1.1 to  $2.8 \mu$ , with a usual width of about  $0.5 \mu$ . The chromosome formula may be tentatively stated as 2 (5A + 3B + 3C). One pair of type A chromosomes are long, all the other pairs are short. Two pairs of type B are fairly long, the third pair is short. Of type C, one pair is fairly long, the other two pairs are short. No satellite chromosomes were observed.

L. repens Bart. 2n=20 (fig. 9).—The chromosomes of this native species appear somewhat thicker than in most other species, and constrictions are not evident in all cases. The following formula is therefore only tentative: 2(4A+3B+2C+D). Of type A there are two pairs of medium length, and two short. Two pairs of type B are of medium length, and one pair is long. Both pairs of type C are long. The single satellite chromosome shown in figure 8 is nearly as long as the longest and has a submedian constriction, the large satellite being attached to the longer arm. Both satellites were observed in another case. Chromosome lengths vary from 1.5 to 3.0  $\mu$ .

L. sericea Benth. n=9 (fig. 10).—This count was taken from division figures of the microspore nuclei, confirming the diploid count of 18 by Cooper. As figure 10 suggests, not much can be said about the morphology of the chromosomes since they are unusually contracted, probably in consequence of fixation in Carnoy's alcohol-acetic solution. It is possible, however, to recognize two or three chromosomes of type A, several of type C, and at least one of type B. None of type D were observed. A few first and second division figures were seen but were too crowded for an accurate count.

L. capitata Michx. 2n = 20 (fig. 11).—Seeds of this native species were obtained from plants collected at several stations in Dane County. Since as many as eight varieties have been described, it was thought that a study of the chromosome complements of two of the most divergent types might aid in evaluating such varietal differences. One type had ovate, very pubescent leaves, the other lanceolate glabrous leaves. Although seeds from cleistogamous fruits only were sown, the plants obtained from them showed considerable variation from the parental types, indicating a probable high degree of heterozygosis. The somatic chromosome number was found to be the same in both forms. The chromosome morphology also is practically identical in the two. Again at least four pairs of type A chromosomes are distinguishable; here they are of varying lengths. Of type B only one pair could be identified with certainty; two or more pairs of type C, one pair long and one of intermediate length. A single long chromosome of type D, with a small satellite, was recognized (fig. 11). The chromosome lengths varied from 1.8 to 2.8  $\mu$ .

L. variegata Cambess. (L. juncea Pers.). 2n = 20 (fig. 12).—This number is presented tentatively,

four clear equatorial plate figures having been drawn and studied. Since it does not agree with the number previously reported by Cooper, additional plants might well be studied to confirm one or the other count. It may be obvious to point out that the number of chromosomes in a given figure may be variously interpreted by different workers. For example, an apparent end-to-end association of two chromosomes may in reality be a single chromosome with a deep constriction. This difficulty of interpretation often appears in species of Lespedeza even in otherwise favorable preparations. The specific appellation itself suggests the possibility of the existence of races with different chromosome numbers. A study of the chromosome morphology seems to show the following formula: 2 (3 or 4A + 2 or 3B+3C+D). The chromosomes of type A seem mostly short, although one pair is of medium length. Those of type B are of medium length. Of type C two pairs are long and one pair is of medium length. The chromosomes of the single pair of type D appear short in figure 12, but in another plate seen they were clearly of medium length. Choromosome lengths vary from 1.2 to 3.0  $\mu$ .

 $\bar{L}$ . hirta (L.) Hornem. (= L. villosa Pers.). 2n=20 (fig. 13).—The chromosomes are as large as those of any species studied, with lengths ranging from 1.6 to 3.3  $\mu$ , and with an average width of 0.7  $\mu$ . The chromosome formula may be expressed: 2 (4A + 3B + 2C + D). Type A consists of two long pairs and two of medium length; type B, of two long pairs and a pair of medium length; of type C, a short pair and a long pair can be identified; a single short pair of type D is present, with no evident constriction.

tions and bearing minute satellites.

L. pilosa Sieb. et Zucc. 2n=20 (fig. 14).—In contrast to the last named species, the somatic chromosomes of this one are small, ranging from 1.5 to 2.6  $\mu$  in length, and widths of only about 0.3  $\mu$ . The chromosome formula is 2 (4A + 2B + 3C + D). Type A includes a long pair, two pairs of medium length, and one short pair; type B, one long pair and one of medium length; type C, one long pair, one of medium length, and a single short pair. The chromosomes of the single pair of type D are as short as any in the complement, if they are not the shortest; their satellites are of medium size.

Section Campylotropsis Bge.—L. macrocarpa Bunge. 2n=22 (fig. 15).—The chromosome complement of this species appears distinctive in that nearly half of the chromosomes are less than  $2 \mu$  long. However the range of lengths is about the average for the genus, varying from 1.5 to 3.0  $\mu$ . Widths are relatively high, averaging  $0.6 \mu$ . Chromosome morphology therefore is not very distinctive, but at least a few of each type may be distinguished. There seem to be four pairs of type A chromosomes, one long pair and three short. Three chromosomes resemble type B, indicating that at least two pairs of medium length are present. Type C is represented by at least two pairs, both long. The single chromosome of type D seen seems to have

a median constriction and bears a rather small satellite.

Section Microlespedeza Maxim.—L. striata var. Kobe 2n=22 (fig. 16).—This variety was introduced from Japan by J. B. Norton in 1919. Chromosome size appears to be rather small, with lengths varying from 1.3 to 2.3  $\mu$ . The morphology appears distinctive enough to present a formula: 2 (4A +4B+3C). All four pairs of type A chromosomes are short (1.5  $\mu$  or less). Three pairs of type B are short, and one pair is long. Two pairs of type C are long, the other pair being of medium length. No satellite chromosomes were observed.

L. stipulacea Maxim., although listed as synonymous with L. striata Hook. et Arn. by the Index Kewensis, would appear to be a valid species on the basis of the chromosome number (2n = 20) as given

by Cooper.

Section Uncertain.—The following species have not been assigned to any section of the genus, and lack of flowering material has as yet prevented any attempts to place them. In a few cases a morphological similarity to species mentioned above has been noted.

L. procumbens Michx. 2n = 20 (fig. 17).—This native species, because of its similarity to L. repens, should probably be included in the subsection Eulespedeza. Chromosome size in general seems to be intermediate, with a range of length from 1.6 to  $2.9 \,\mu$ . The chromosome formula may be expressed: 2 (4A + 3B + 2C + D). Three pairs of type A chromosomes are of intermediate length or slightly longer, and one pair is short. Two pairs of type B are long, and one pair is of medium length. The single type D chromosome shown in figure 17 is short; its satellite is of medium size.

L. frutescens (L.) Britton. 2n = 20 (fig. 18).— This species also is native to the United States and resembles L. procumbens in both size and form of chromosomes. Here, however, although the chromosome formula is the same, two pairs of chromosomes of type A are long, and two pairs are somewhat shorter. Of type B there are one long pair, one short, and one of intermediate length. One pair of type C is long, one pair short. The two chromosomes of type D are long, with approximately submedian constrictions and satellites of medium size.

L. latissima Nakai. 2n = 20 (fig. 19).—Very little appears to be distinctive about the somatic chromosomes of this Asiatic species. Chromosome lengths range from 1.6 to  $3.5 \mu$ . Positions of constrictions indicate that the formula is: 2 (3A + 3B + 3C + D). The three pairs of type A chromosomes are, respectively, long, medium, and short. Those of type B and C show a similar range of lengths. The single pair of type D is short, with probably median constrictions and large satellites.

L. inschanica (Maxim.) Schind. 2n = 20 (fig. 20).—The chromosomes of this species resemble closely those of L. latissima. Lengths show a slightly different range, from 1.3 to 3.0  $\mu$ . The approximate formula is 2 (3 or 4A + 2 or 3B + 3C + D). The

chromosomes of type A are all long, those of type B all of intermediate lengths; of type C, two pairs are short and one pair is of medium length. The two type D chromosomes are of medium length with fairly small satellites.

L. Maximowiczii Gandog. (= L. bicolor Turcz.). 2n=22 (fig. 21).—Chromosome lengths vary from 1.5 to 2.6  $\mu$ . There appear to be more chromosomes of type C than usual, as shown by the formula: 2(3A+3B+4C+D). Range of lengths: Type A, two long pairs and one of medium length; type B, three pairs of medium length; type C, three short pairs and one of medium length; type D, one fairly long chromosome seen, with a submedian constriction and a small satellite.

L. Thunbergii Nakai. 2n=22 (fig. 22).—The chromosome formula for this species is 2 (4A+3B+3C+D). There appear to be one long pair of type A, one of medium length, and two short pairs. Of type B two pairs are long and one pair is intermediate in length. Those of type C show a similar range of lengths. Only one chromosome of type D is identifiable in figure 22. It is of medium length, with a median constriction, and bears a fairly large satellite. Chromosomes vary in length from 1.1 to  $2.6 \mu$ .

L. virginica (L.) Britton. 2n = 22 (fig. 23).— This is the only American species studied whose somatic chromosome number is other than 20. Its chromosome formula is 2 (4 or 5A + 3 or 4B + 3C). There are two or three long pairs of type A chromosomes, and two short pairs. Of type B there are two or three long pairs and one pair of medium length. The three pairs of type C are, respectively, long, medium, and short. No satellite chromosomes were observed. Lengths of chromosomes vary from 1.2 to  $3.0 \mu$ .

L. japonica var. intermedia (Nakai) Nakai. 2n = 22 (fig. 24).—The chromosome formula is 2 (4A + 3B + 4C). Of the type A chromosomes, one pair is long, two are short, and one is of intermediate length. The three pairs of type B are of medium length. Three pairs of type C also are of medium length and one pair is long. No satellite chromosomes were observed. Range of lengths, 1.3 to 3.1  $\mu$ .

L. robusta Nakai 2n = 22 (fig. 25).—Although observations of this species to date have been limited to fairly young plants, the size and shape of the leaves, as well as the habit of growth, would suggest that it is a member of the subsection Macrolespedeza. The chromosome morphology is similar to that of several other 22-chromosome species, the formula being the same as in L. cyrtobotrya and L. Thunbergii: 2 (4A + 3B + 3C + D). Here the type A chromosomes are represented by two long pairs, one of medium length, and one short. Of type B, two pairs of medium length, and one pair is long. Of type C, also, there are two pairs of medium length and one long pair. The two of type D are of medium length with no evident constrictions, and bear medium-sized satellites. Chromosome lengths vary from 1.3 to 2.7  $\mu$ .

L. daurica Schind. 2n = ca. 44 (fig. 26).—This Asiatic species is of interest in that it is the only one of the genus which has been shown to be tetraploid. It is to be noted that there is a considerable discrepancy between the count here given and that reported for this species by Cooper. For this reason counts were made from root tips taken from 8 or 10 individual plants. It appeared from a study of a considerable number of figures that the chromosome number in these plants must be either 42 or 44. The higher number is probably the correct one. In view of the wide discrepancy in counts it is possible that races occur with different chromosome numbers.

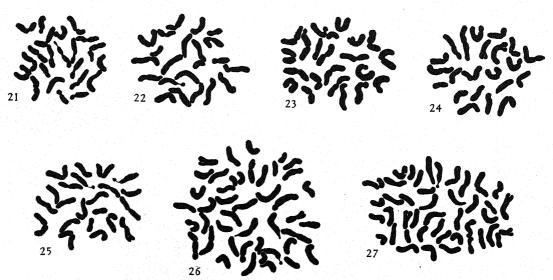


Fig. 21-27.—Fig. 21. L. Maximowiczii (2n = 22).—Fig. 22. L. Thunbergii (2n = 22).—Fig. 23. L. virginica (2n = 22).—Fig. 24. L. japonica var. intermedia (2n = 22).—Fig. 25. L. robusta (2n = 22).—Fig. 26. L. daurica (2n = 21).—Fig. 27. L. daurica var. shimadai (2n = 21).

There is no correlation between chromosome numher and plant or organ size, since several members of the section Microlespedeza are considerably larger in both plant and organ size than is L. daurica. Nuclear and cell size do appear to be somewhat larger than in the other species, as might be expected. The chromosomes are similar in general size and morphology to those of the diploid species. Lengths vary from 1.0 to 3.0 \(\mu\). Since constrictions were not evident in many of the chromosomes, no attempt is made to present a formula. However, all the various types of chromosomes may be identified. There appear to be about 8 pairs of chromosomes of type A. In no figures examined was more than a single pair of type D chromosomes found. They appear to be of medium length, with satellites of medium size. In one case a division figure with about twice the usual number of chromosomes was observed in the dermatogen of the root.

L. daurica var. shimadai (Masamune) Masam. and Hosokawa. 2n = ca. 44 (fig. 27).—This variety differs only slightly in leaf shape from the typical form of the species. An examination of figure 27 will show that in the chromosome size and morphology also, the type and variety are practically identical.

Discussion.—The chromosome numbers of approximately one-fourth of the known species of Lespedeza have been determined. The number of species studied is therefore sufficient to warrant suggestions as to the basic numbers for the genus or its respective sections. Table 2 summarizes the reports to date on the chromosome numbers in the genus. In three cases, conflicting numbers were previously reported, but they are omitted from the table.

TABLE 2.

Haploid number	Number of speci and varieties	es Percentage of total
9	5	16.1
10	13	41.9
11	11	35.4
22	2	6.4
	<del></del>	
	31	99.8

Nine of the seventeen species listed by Pieters as native to the United States have a haploid count of 10, one species only (L. virginica) having the count n=11. Of the remaining species, most of which are Asiatic, only about 12 per cent (24) have been studied. Of these species 13 have the haploid number 11, 3 have 10, and 5 have 9. (L. bicolor and L. cyrtobotrya, previously reported by Kawakami as n=9, were found to be 2n=22 by the present author. Similarly, L. variegata, reported by Cooper as having 2n=18, appears to have 2n=20.)

It seems likely, therefore, that the basic number for the American species of Lespedeza is 10. For the rest of the genus it may well be that the basic number is 11, although such a conclusion would necessarily be based on rather limited data.

For the genus as a whole it would appear that an increase or diminution in chromosome number of the haploid set by one or two (perhaps in consequence of fragmentation or translocation) might account for the occurrence of such closely similar numbers. The low frequency of the number 9 may indicate a derivation from one of the other numbers.

It appears doubtful whether, as suggested by Senn, chromosome numbers alone can aid greatly in distinguishing species of Lespedeza from those of Desmodium. The somatic numbers of only three species of Desmodium (in each case 22) have been determined to date. Yet, if subsequent work shows that the number 22 is of constant occurrence in that genus, it may be significant that of the American species of Lespedeza examined cytologically, all but L. virginica have the diploid number 20.

The chromosomes vary but little as to number. size, and form among species or even among sections of the genus. The single species of the section Campylotropsis studied, L. macrocarpa, has the diploid number 22. The single species (L. striata var. Kobe) of the section Microlespedeza has the diploid number 20. The few species studied in the subsection Macrolespedeza appear to be constant as to number (2n = 22), and in general as to chromosome size. Within the other sections or subsections of the genus. however, variations in number occur. Satellite chromosomes sometimes show what may be specific differences in length, in location of constriction, and in size of satellites. Coonen (1938) has noted similar variations in satellite size and in length of setae in species of Ranunculus.

The apparent presence of a single pair of satellite chromosomes in *L. daurica* may indicate that this is an allotetraploid species derived originally from a cross between a 22-chromosome species with a pair of satellites and one without such a pair or with a pair which has lost its satellites.

### SUMMARY

The chromosome number has been determined for 22 species and three varieties of the genus Lespedeza. In four out of five species whose numbers have previously been reported, the counts disagree with those here given.

In the section Archilespedeza, subsection Macrolespedeza, L. bicolor, L. cyrtobotrya, and L. cyrtobotrya var. pedunculata have the somatic chromosome number 22. In the subsection Eulespedeza the following chromosome number determinations were made: L. sericea, n=9; L. violacea, L. Stuvei, L. repens, L. capitata, L. variegata, L. hirta, and L. pilosa, 2n=20; L. floribunda and L. virgata, 2n=22.

L. macrocarpa, in the section Campylotropsis, has the number 2n = 22. In the section Microlespedeza, L. striata var. Kobe has the number 2n = 20.

The remaining species and varieties investigated have not, up to the present time, been assigned to

any section of the genus. The chromosome numbers are as follows: L. procumbens, L. frutescens, L. latissima, and L. inschanica, 2n = 20; L. Maximowiczii, L. Thunbergii, L. virginica L. japonica var. intermedia, and L. robusta, 2n = 22; L. daurica and L. daurica var. Shimadai, 2n = ca.44.

It is suggested that the basic chromosome number for the American species of the genus is 10. For the rest of the genus, on the basis of work to date, it appears likely that the basic number is 11, although n=9 has been reported for certain Japanese species.

The somatic chromosomes are small and fairly similar in size and form as among species and even sections of the genus. The chromosomes display constrictions in median, submedian, and subterminal positions. Chromosomes of these types appear in varying proportions in all species studied. Satellite chromosomes sometimes appear to show specific differences in length, position of constriction, and in size of satellites.

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## THE SIGNIFICANCE OF CERTAIN ABNORMALITIES IN EQUISETUM 1

### Robert H. Tschudy

Abnormality and irregularity in this genus are by no means uncommon. Many workers, including Schaffner (1927, 1928, 1929, 1933), Kashyap (1930), and Allen (1928), have described and listed some of these. I have found two additional types of abnormality which I hope will help to clear up some of the differences in opinion relative to an interpretation of the cone and sporangiophore structure insofar as they relate to problems of structure or phylogeny.

IDEAS CONCERNING THE CONE AND SPORANGIO-PHORE STRUCTURE OF EQUISETUM.—There are two schools of thought concerning the structure of the cone of Equisetum. One, championed by Browne (1912, 1915, 1920, 1921, 1923, 1927, 1933) and others, contends that the cone is made up of a shortened axis consisting of nodes and internodes and that the position of the sporangiophore designates the node. This is the more widely accepted view. Opposed to this is Barratt (1920) who contends that the arrangement of the xylem strands and the mode of origin of the sporangiophore traces fail to support the view that the fertile axis is made up of alternating nodes and internodes.

The theses concerning the nature of the sporangium-bearing organ are more numerous and varied. Eames (1936) indicates that the structure has been called a lateral branch bearing a whorl of sporangia; the fertile lobe of a dorsiventrally divided sporophyll, the ventral lobe of which is either lost or is also fertile; the stalk of a raised and divided sporangium; a sporophyll; an organ sui generis.

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To obtain an idea of the ratiocination behind the possibilities, we must consider the situation among the fossil progenitors of the group. Present-day Equisetae were most certainly derived from the almost identical Equisetites of the Mesozoic. These in turn probably were derived from the Calamites or their forerunners. One of the difficulties of interpretation of the sporangiophore and cone structure of present-day Equisetae lies in the presence of bracts in the Calamitaceae of the Coal Measures and the absence of bracts in Equisetum. What became of the bracts in the Calamarian cone?

When we consider the fossil representatives in the order of their antiquity, we find the oldest known ancestor of the group, Archaeocalamites, in the upper Devonian strata. This genus bore cones and whorls of peltate sporangiophores very similar to those found in present-day Equisetae. Bracteate cones with one or two exceptions do not occur below the Coal Measures and the Permian strata. Here are found two examples of Calamitaceae which should be mentioned. One is Calamostachys which bore fertile shoots consisting of whorls of peltate sporangiophores alternating with intermediate and equidistant verticils of sterile bracts. The second is Palaeostachya which bore sporangiophores not inserted midway between sterile bracts, but in the axil of each verticil of bracts. However, the xylem trace of the sporangiophore in this genus traversed the internode for some distance and then was reflexed downward almost to its point of origin before entering the sporangiophore.

Smith (1938) is of the opinion that in presentday Equisetae the annulus is all that remains of the Calamostachys type of bract-in other words, that the bracts have become lost. He is very definite concerning the non-foliar nature of the sporangiophore. Browne (1927) postulates that the Calamarian cone is a modification of the Archaeocalamitean bractless cone and that the type of greatest antiquity persists to the present-day in Equisetum. Thus the Equisetaceae are not to be considered direct line descendants from the Calamites, but a very early offshoot. The possibility that the Equisetaceae were derived from a Sphenophyllaceous ancestry lies in the assumption that the sporangiophore is part of a dorsiventrally bilobed sporophyll. The more recent Sphenophylls had highly complex cones bearing peltate sporangiophores on one or both lobes of a dorsiventrally bilobed leaf. There is little evidence from a study of the anatomy of the cone (Browne, 1927) or of the sporangiophore to indicate that the sporangiophore of Equisetum has arisen, during phylogeny, from the union of the dorsal and ventral lobes of a sporophyll. Browne (1920) has shown that the anatomy of the sporangiophore indicates that it is a whole organ. She has also shown that two successive whorls cannot be considered as two lobes of a bilobed leaf.

The above considerations give us little insight into the actual nature of the fertile organ. In my opinion, the view held by Barratt (1920) that the present-day sporangiophore is an organ sui generis has little to support it. The evidence I propose to submit indicates that the sporangium-bearing organ is a modified leaf.

Abnormalities found in Equisetum telmateia ehrh.—These abnormalities are of two different types. The first, found only once, is an apparent phyllody of several whorls of sporangiophores occurring midway on an otherwise normal cone. The second type consists of intercalary cones or cones having the vegetative axis resume growth and develop to a greater or less extent above the cone. This type is so common that twenty specimens have been collected on an afternoon's walk on the campus of the University of Washington.

Phyllody of sporangiophores in Equisetum, to my knowledge, has never been reported. In the single cone in which it occurred (fig. 1, 3) there was no evidence of mechanical, insect, or fungal injury. The first five "nodes" above the annulus bore normal sporangiophores with normal sporangia. A region made up of from four to five "nodes" was abnormal, bearing no sporangia on the more or less filiform appendages. Some of these appendages were short and stubby, appearing much like the stalk of a sporangiophore without the enlarged cap-like sporangium bearing tip. Others were somewhat swollen at the tip, but completely sterile. Still others were long and tapered growing for a distance at right angles to the cone, then bending upward. Some of these latter were united at the base, giving somewhat the appearance of a segment of a leaf sheath. Above this region the cone bore normal sporangiophores and normal sporangia.

Intercalary cones have been reported only once by Kashyap (1930). He found one specimen of E. debile bearing such a cone. Intercalary cones, usually found in the upper one-third of a vegetative branch, consist of from two to a dozen or so more or less well-defined whorls of sporangiophores. Of the forty or fifty intercalary cones that I have collected, those that are made up of but two to four nodes are the most interesting. They are without exception abnormal to some degree and tend, more than the larger cones, to elucidate the nature of the sporangiophore (fig. 8, 18).

Figure 8 shows a cone which appears to lack an annulus. The lowest node shown consisted of a large leaf sheath which completely obscured the three nodes immediately above it. This has been cut away to show the structures. Possibly there is only a partial annulus as evidenced by the apparent free lobes intermediate between the full leaf sheath and the first complete whorl. The two lobes or expanded structures to the left are sterile, while the two to the extreme right are cylindrical appendages bearing on or near their apices a thickened expansion to which sporangia are affixed. The lowest complete fertile whorl is made up of sporophylls strikingly similar to those seen in figure 18. Though somewhat aberrant, they are more nearly peltate than those of the node next above. There are comparatively few sporangia present on this node (the second from the top) and as a whole it takes on somewhat the appearance of a distorted, split-up leaf sheath. The uppermost node figured is almost normal. Branches penetrate through its base in a normal fashion, although a complete whorl of branches is absent. The sheath itself is split down one side to a point slightly below its point of attachment to the node.

Nodal nature of cones.—The arrangement of the sporangiophore whorls in relation to vegetative nodes is indicated in figure 2. The sporangiophores are crowded into about eight rows on a little more than two-thirds of the perimeter of the stem and absent on the remainder. In their stead leaves, some fully an inch long, arise from a continuation of the sporangiophore whorl.

The nodal nature of an intercalary cone is also shown by the arrangement of tissues in a longitudinal section (fig. 7). This is a median section through a cone similar to that drawn in figure 18. The vascular supply, as well as the nodal septae and even the lignified so-called nodal rings which are present in normal nodes, are shown. The lack of nodal septae and the irregularity of departure of traces in the normal cone led Barratt (1920) to conclude that the cone axis is not made up of nodes and internodes. I am aware that the structure and arrangement of elements in an intercalary cone of only three nodes cannot prove the nodal condition of terminal cone axes. However, the gradation from intercalary cones consisting of one or two nodes to almost normal appearing intercalary cones made up of many nodes.

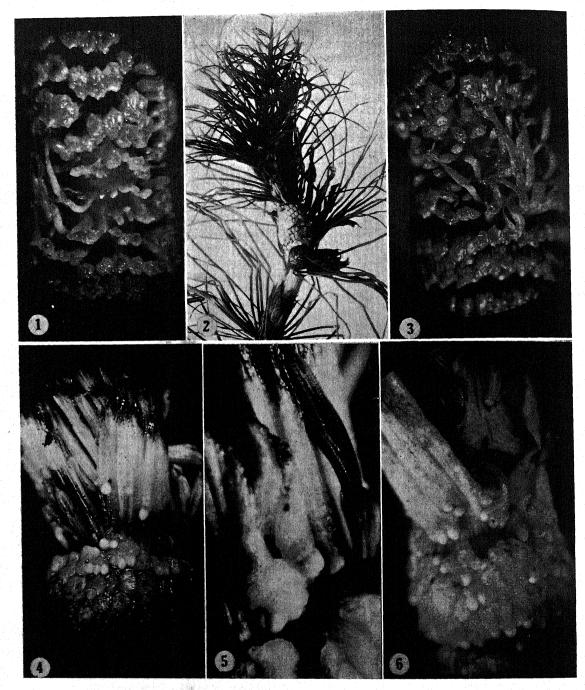


Fig. 1-6.—Fig. 1. An enlarged photograph showing the phyllody or sterility of a segment of an otherwise normal cone.—Fig. 2. An intercalary cone which bears normal leaves as continuations of the fertile nodes. The cone is completely sterile on the side which bears the leaves.—Fig. 3. Another view of the same cone as seen in fig. 1.—Fig. 4. An intercalary cone showing sporangia on the back of the first sheath of leaves above the cone.—Fig. 5. An enlargement of several sporophylls bearing ligulate appendages.—Fig. 6. Another intercalary cone bearing sporangia at the base of the first leaf sheath above the cone. Note that in many cases the sporangia in the cone proper are not covered by the growth of the sporophylls.

coupled with the occasional phyllody of part of a fertile node, are strongly presumptive evidences that the sporangiophores are borne at nodes. The sporangiophores.—Figures 9 to 16 have been arranged to show a complete series of sporangiophores ranging from an almost normal quasi-peltate

sporangia on the outer surface. The drawings were made with the aid of a camera lucida and in most cases show both the inner and outer surfaces of the

sporangiophores.

Figure 9 is the most nearly normal. It is peltate, but the stalk is somewhat more robust than normal and the top surface is irregular and furrowed and not raised, swollen, and angular as are entirely normal sporangiophores. Figure 10 shows the lateral union of at least two sporangiophores bearing many sporangia. A short leaf-like protuberance is present as an extension of the top of the sporangiophore, indicating that practically the whole leaf or segment of a whorl of leaves has been reduced to sporangiophore. Broad sporangiophores of this type often possess two or more vascular strands. Figure 11 is an example of a further extension of the leaf or less reduction of leaf to sporophyll. Here the sporangia appear to be borne on the inner or adaxial surface of the leaf. A photograph of this type of sporangio-

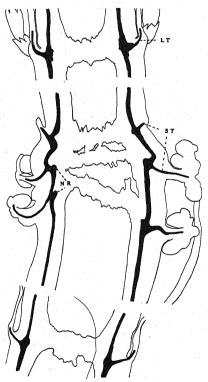


Fig. 7. Longitudinal section through an intercalary cone and adjoining stem. Vascular tissue in black. The long internodes have been omitted and are indicated by the breaks. nr, nodal rings; lt, leaf trace; st, sporangiophore trace.

phore is shown in figure 5. Figure 12 is practically a counterpart of figure 11, except for the fact that the ligulate extension of the leaf is more pronounced and the number of sporangia in relation to the size of the sporangiophore is much reduced. Figure 13 is an almost sterile segment of a supra-annular whorl of sporangiophores. This segment is, in appearance,

very like a segment from an abnormal annulus. In figure 14 the single sporangium is borne on the outer or abaxial surface of the leaf-like appendage. It is well known that the normal sporangia initials arise at the top of the young peltate appendage and, by

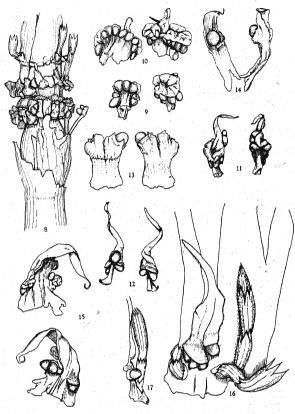


Fig. 8-17.—Fig. 8. An intercalary cone of two nodes and an abortive annulus.—Fig. 9-13. Inner and outer views of sporophylls and their gradation into leaf segments as the number of sporangia decreases.—Fig. 14. Sporangium borne on outer surface of leaf, and three sporangia.—Fig. 16-17. Sporangiferous leaf segments through whose bases lateral branches have penetrated.

the subsequent growth of tissue between them, are eventually pushed around underneath the sporangiophores' protecting edges, with the final appearance of being borne on the adaxial surface.

That the origin of sporangia on normal and abnormal or leaf-like sporangiophores is identical may be inferred from the above. A lateral view is given of this sporangiophore, showing how prominent the adaxial sporangia appear when they arise near the center of a sporangiophore rather than near its edge. The same phenomenon is observed in figure 15, a much larger, more leaf-like example. Here the sporangia are borne near the edges of the leaf but definitely on the outer surface. An extreme example of phyllody of sporangiophores is shown in figure 16. Here the sporangia are borne on the back of the leaf. At the point of insertion of the sporangium there is a swelling, while above the sporangium the leaf seg-

ments are somewhat reduced in size. The presence of sporangia on almost normal or normal leaf segments is often encountered on the first whorl of leaves above an intercalary cone (fig. 4, 6). In some cases lateral branches may be seen penetrating the base of the sheath, while in others the branches may be absent or, due to the looseness of the sheath, may grow up inside the sheath. Figures 16 and 17, and less clearly figure 4, show the penetration of branches through the fertile leaf segments. On one sheath (fig. 16) four sporangia are seen subtending an apparently stunted portion of the leaf. It will be noted that the sporangia are inserted above the level



Fig. 18. An intercalary cone bearing two nodes of abnormal sporangiophores and normal sporangia. The annulus is partly sporangiferous.

at which the branches penetrate the leaf sheath. In no case, even when sporangia and branch were in proximity, was there the slightest evidence that sporangia are ever borne on lateral axes.

The non-commital term "sporangiophore" has been given to the fertile organ in Equisetum because morphologists have been unable to agree on the nature or origin of this structure. In view of the evidence cited above, I propose that the more descriptive and accurate term "sporophyll" be used.

The annulus.—Some workers are willing to concede that the annulus is a reduced whorl of leaves, yet would not be willing to ascribe to the supraannular whorls the same derivation. I can find no reason for concluding that the supra-annular whorls are any different from the annulus, particularly in the abnormal intercalary cones that I have examined.

Several examples have been seen of a sporangiferous annulus bearing many vascular strands which are continuous with the vascular system of the stem and which end at the base of the sporangia. Barratt

(1920) states that the lack of vascular supply to the annulus, or even to abnormal fertile annuli, is an argument against the foliar nature of the annulus. This argument has been discounted by Browne (1920) and by the present observations.

Figure 18 shows an intercalary cone made up of two nodes and a so-called annulus. The annulus is partially but not completely sporangiferous. When the cone is reduced to the extent seen in this figure and figure 8, the normal appearing peltate sporophyll is universally absent. Some of the appendages are sterile; some bear sporangia on their abaxial face. Sporangia are not at all hidden but appear as excrescences in almost any position.

When an annulus is partly sporangiferous, the leaf sheath at the next node below is often modified. The portion of the leaf sheath below the sterile part of the annulus is normal, while the portion directly below the fertile part of the annulus is often reduced, sometimes to the extent of appearing no different from an annulus itself.

THEORETICAL CONSIDERATIONS.—An examination of the abnormalities figured shows that as the number of sporangia per sporophyll decreases, the more leaf-like the sporophyll tends to become. Is this lack of growth of the expanded leaf due to a lack of food? Do the sporangia have some mechanism for commanding the food supply to their exclusive use, or does the sporangium or its contents produce some substance which on diffusing from the sporangium produces a thickening of the sporophyll—a proliferation of tissue? It is a fact that an increase in the number of sporangia in general produces an increase in thickening and a more flattened peltate structure, tending to push the sporangia laterally and inward, resulting in a certain amount of protection to the sporangia as well as producing a decrease in the foliar appearance of the sporophyll. Support for the implication that either the food supply is commandeered or that a substance diffuses from the sporangia is found when an examination of the intercalary cones is made, for when the sporangia are borne on the leaves of one whorl, the sporophylls below are more nearly normal than they are on the first whorl (fig. 6). Does the cone or fertile segment produce some substance which diffuses downward to form the annulus or, more precisely, to inhibit the growth of leaves and branches from this node and the node below? A consideration of the situation present in Equisetum sylvaticum makes the query sound less fantastic, for here the fertile stem develops branches only after the cone has shed its spores and dried up. A careful examination of Equisetum telmateia in the field shows that here also a great many fertile branches become vegetative after the cone has shed its spores.

### SUMMARY

A discussion of the theories concerning the origin of the cone and sporangiophore of Equisetum is given.

Two types of abnormal cones found in Equisetum telmateia Ehrh. are discussed—i.e., the phyllody of a segment of an otherwise normal cone and the occurrence of intercalary cones midway on an otherwise vegetative stem.

The nodal nature of the intercalary cones was demonstrated by a section through one of them showing nodal septae and by one which developed sporophylls on one side of the stem and sheath leaves at each node on the other side.

A complete series of sporophylls ranging from normally peltate ones to very much expanded normal sheath leaves bearing but one or two sporangia is figured. This series demonstrates that the sporangia arise on the outer surface of the sporophyll and are pushed around to the inner by the growth or hyperplasia of the cells immediately surrounding them. As more sporangia are produced, the hyperplasia increases, at the same time decreasing the amount of leaf-like appendage until in the ultimate condition the ligulate appendage is lost and the sporophyll takes on the peltate appearance.

It is suggested in referring to the sporangium bearing structure in *Equisetum* that hereafter the term "sporophyll" be used in place of the non-committal, less exact term "sporangiophore."

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# EARLY ROOT AND SHOOT GROWTH IN TWO VARIETIES OF AVENA SATIVA IN RELATION TO GROWTH SUBSTANCES $^{\rm 1}$

## Samuel Kaiser and Harry G. Albaum

GROWTH DIFFERENCES between varieties and species which have or are presumed to have a simple genetic basis constitute an excellent field for the study of the manner in which certain genetic factors operate in producing their effects. Such differences have been investigated by van Overbeek (1935), Lehmann (1936), Hinderer (1936), Graze and Schlenker (1936), and others; the importance of studying them has been repeatedly emphasized by Sinnott (1939). The present contribution attempts to add to our knowledge on this subject by presenting pertinent data concerning the growth of roots and shoots in two varieties of oats and the effects of a synthetic growth substance (3-indole acetic acid) on their growth.

MATERIALS AND METHODS.—The two varieties of Avena sativa used in these experiments, Black Norway and Fulghum, were obtained through the cour-

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tesy of Dr. G. M. Reed of the Brooklyn Botanic Garden. The oats were dehusked, soaked for two hours in tap water at room temperature, and illuminated for several hours. They were then germinated for twenty-four hours in an incubator maintained at a temperature of 25°C. At the end of this time, the young seedlings were transferred to the test solutions in 400 cc. beakers. The beakers were first lined with filter paper and received 50 cc. of the test solutions (tap water in the control beakers) which the filter paper absorbed. Twelve to fifteen young seedlings were carefully inserted between the filter paper and the glass of each beaker (fig. 1), the beakers were covered with Petri dish covers and transferred to the incubator or left uncovered and placed in a light-proof humid glass chamber. Under these conditions, the seedlings continued their growth, the primary and secondary roots growing straight in most cases so that repeated measure-

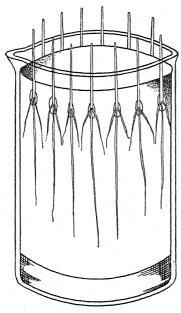


Fig. 1. Sketch showing method of following the growth of oat seedlings.

ments could be made through the glass without difficulty and without disturbing the plants. The length of the longest root was used as a criterion of total root growth (Lane, 1936). Shoot measurements were taken from the point of emergence from the grain to the coleoptile tip and thus included the length of the mesocotyl as well as the coleoptile. (All figures are averages of at least ten plants.) Final measurements on the length of shoots and number of

Table 1. Effect of different concentrations of 3-indole acetic acid on the length of the longest root (in mm.).

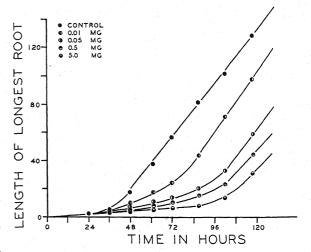
Hours after					
germi-		• 0.01	0.05	0.5	5.0
nation	Control	mg./l.	mg./l.	mg./l.	mg./l.
	I	Black No	rway		
24	3.0	3.0	3.0	3.0	3.0
36	5.6	5.0	4.3	3.2	3.3
48	17.8	10.6	7.8	5.2	3.3
61	37.7	17.5	11.4	8.3	5.6
72	56.8	24.5	14.4	10.8	6.3
87	81.1	43.9	20.7	15.2	8.1
102	101.3	71.2	32.9	23.6	13.8
118	128.8	97.3	58.7	44.3	31.1
		Fulghu	m		
24	2.5	2.5	2.5	2.5	2.5
36	4.6	4.4	3.9	3.9	3.1
48	14.3	7.9	5.8	5.2	4.9
61	31.9	13.9	10.0	8.0	6.3
72	46.6	19.0	12.3	9.9	6.8
87	62.9	34.9	17.9	14.5	7.7
102	83,2	56.6	30.3	23.7	8.6
118	99.3	79.8	51.1	41.3	11.6

roots were made at the close of each experiment by removing the plants from the beakers.

The stock auxin solution was prepared by dissolving 3-indole acetic acid (Eastman Kodak Co.) in distilled water with slight heating. The concentrations employed in these experiments, made by diluting the stock solution, ranged between 0.002 mg./l. and 50.0 mg./l.

RESULTS.—Root growth.—Normal growth for the roots of the two varieties under the conditions of these experiments is represented by the figures in table 1 (control column). It will be noted that Black Norway grows more rapidly than Fulghum. The average rate of growth (final length of the longest root divided by the growth period) is 1.09 mm./hr. for Black Norway and 0.84 mm./hr. for Fulghum. In another experiment the figures were 1.05 for Black Norway and 0.85 for Fulghum.

Shoot growth.—The normal growth measurements on the length of the young shoot (mesocotyl



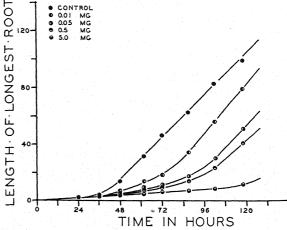
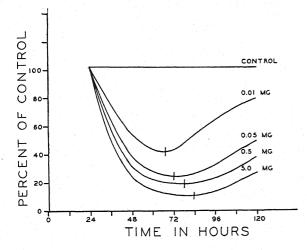


Fig. 2A (above). Effect of different concentrations of 3-indole acetic acid on the length of the longest root in Black Norway.

Fig. 2B (below). Effect of different concentrations of 3-indole acetic acid on the length of the longest root in Fulghum.

plus coleoptile) in the two varieties are shown in table 4 (control column). Shoot growth is seen to be slower in Black Norway than in Fulghum (0.24 mm./hr. as compared to 0.36 mm./hr.). A negative correlation thus exists between the rate of root growth and the rate of shoot growth in these two varieties.

Effects of auxin treatment; 1. Root length.—The results in a typical case of the treatment with 3-indole acetic acid on root growth using concentrations from 0.01 mg./l. to 5.0 mg./l. are given in full in table 1 and are shown graphically in figures 2A and 2B. It will be noted that in both varieties all concentrations of the growth substance bring about inhibition for a time, after which the roots begin to grow rapidly again, in all cases approaching the rate of growth for the untreated roots. These facts are more clearly shown when values for each concentration are read from the smooth curves and plotted as percentage of control against time (hours after germination) as shown in figures 3A and 3B. It is ap-



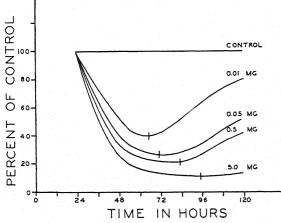


Fig. 3A (above). Relationship between length of longest root expressed as percentage of control and time in hours since germination for Black Norway.

Fig. 3B (below). Relationship between length of longest root expressed as percentage of control and time in hours since germination for Fulghum.

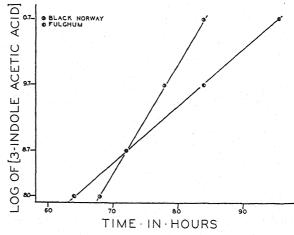


Fig. 4. Relationship between time of maximum inhibition of root length and concentration of applied 3-indole acetic acid.

parent that each of the concentrations inhibits root length maximally at the end of a given time (the percentage of inhibition increasing with increased concentration), after which time the inhibition falls off, each of the curves approaching the untreated control. When the time required to attain maximum inhibition is plotted against the logarithm of the concentration, the straight line plots shown in figure 4

Table 2. Relationship between time of maximum inhibition of root length and concentration of applied 3-indole acetic acid.

Concentration	Time (hours after germination)				
	Black Norway	Fulghum			
0.01 mg./l	. 68	66			
0.05 mg./l	. 72	72			
0.5 mg./l		84			
5.0 mg./l	. 84	96			

are obtained (see data in table 2). The slopes of the two lines are 1.72 for Black Norway and 0.86 for Fulghum.

2. Root number.—The numbers of roots possessed by the two varieties at the end of 112, 158, and 230 hours after germination, when treated with various concentrations of 3-indole acetic acid, are shown in table 3. In this experiment the plants were removed from the filter paper at the end of 112 hours and placed in the glass holders used in the Avena test, the roots continuing their growth in tap water. It will be noted that at 112 hours in the controls, there seems to be little difference in the number of roots in the two varieties. After 230 hours, however, Fulghum possesses a considerably greater number of roots than Black Norway. Treatment with 3-indole acetic acid in both varieties has resulted after 230 hours in an increase in root number of about 100 per cent in the case of the highest concentrations used.

Table 3. Effect of different concentrations of 3-indole acetic acid on root number.

	Averag	ge number o	f roots
Concentration	112 hours	158 hours	230 hours
The state of the s	Black Norwa	y	
0.00 mg./l	3.8	4.0	4.0
0.05 mg./l.		4.0	4.7
0.5 mg./l		4.8	4.8
5.0 mg./l		4.9	5.3
50.0 mg./l		6.3	8.3
	Fulghum		re jar
0.00 mg./l	3.6	4.3	5.2
0.05 mg./l		4.4	5.5
0.5 mg./l		5.3	5.7
5.0 mg./l		5.3	6.2
50.0 mg./l		6.8	10.0

3. Shoot length and coleoptile curvature.—The results of a typical experiment on shoot length of 3-indole acetic acid applied in solution through the

Table 4. Effect of different concentrations of 3-indole acetic acid on the length of the shoot (in mm.).

Hours after					
germi- nation	Control	0.002 mg./l.	0.005 mg./l.	0.02 mg./l.	0.2 mg./l
	P	lack No	rway		
56	4.0	4.9	5.0	5.4	4.7
68	9.9	11.7	12.1	13.0	10.1
99	24.3	28.1	29.2	30.1	32.6
122	29.0	32.5	34.0	37.3	38.9
		Fulghu	m		
56	7.2	8.0	8.1	7.0	6.0
68	17.4	18.2	16.6	16.9	12.3
99	37.7	40.1	36.3	37.0	35.3
122	44.1	44.7	44.0	47.1	45.5

roots are shown in table 4 and are represented graphically in figures 5A and 5B. All concentrations used appear to stimulate shoot growth in Black Norway; the same concentrations, however, have little or no constant stimulatory or inhibitory effect in Fulghum. In order to ascertain whether the coleoptiles of the two varieties respond differently to 3-indole acetic acid applied directly, the standard Avena test was carried out using ten plants of each variety. The experiments on the curvature of the coleoptiles were carried out in a closed glass chamber in the darkroom in which the humidity was high and maintained at a constant level and in which the temperature varied between 23° and 25°C. The technique employed was the same as that described by Went and Thimann (1937) involving double decapitation. The test blocks placed asymmetrically on the coleoptile tips were prepared by soaking sections of 3 per cent agar for twelve hours in a 0.005 mg./l. solution of 3-indole acetic acid. Curvatures were recorded

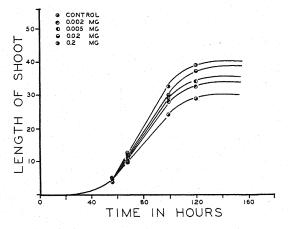
after two hours by the shadowgraph method. The average curvature of the test coleoptiles was 28.5° for Black Norway and 21.5° for Fulghum. A similar determination using a concentration of 0.002 mg./l. 3-indole acetic acid gave an average curvature of 11.1° for Black Norway and 8.9° for Fulghum.

Discussion.—The two oat varieties utilized in these experiments flower at different times of the year when grown in the field. The figures given below were placed at our disposal by Dr. G. M. Reed of the Brooklyn Botanic Garden:

Flowering time

	1929	1930
Black Norway	July 2 June 14	June 25 June 3

An examination of the average growth rates given above for Black Norway and Fulghum shows that there is an inverse relation between the time of flowering and rate of root growth—i.e., the slower the rate of root growth, the earlier the flowering time.



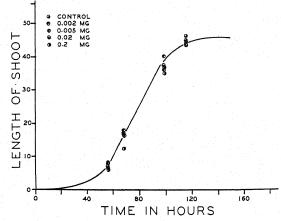


Fig. 5A (above). Effect of different concentrations of 3-indole acetic acid on the length of the shoot for Black Norway.

Fig. 5B (below). Effect of different concentrations of 3-indole acetic acid on the length of the shoot for Fulghum.

Such a relation has been verified for two other oat varieties, Victory and Navarro. If this relation is a general one, rate of early root growth may prove to be a valuable index of flowering time.

With respect to differences in response of roots to the same concentrations of 3-indole acetic acid, the data indicate that both varieties are inhibited to approximately the same degree; 60 per cent for both varieties with the lowest concentration used (0.01 mg./l.), 77 per cent and 74 per cent with a concentration of 0.05 mg./l., 82 per cent and 79 per cent with a concentration of 0.5 mg./l., and 90 per cent for both varieties with the highest concentration used (5.0 mg./l.). Although both varieties are maximally inhibited to the same extent, differences exist between them in the time at which maximum inhibition is obtained. With the exception of the lowest concentration, 0.01 mg./l., where Black Norway seems to recover from the effects of the treatment somewhat later than Fulghum (68 hours after germination in contrast to 66), the latter recovers later for the higher concentrations used.

The difference in the time at which recovery begins may best be expressed by the slopes of the two lines shown in figure 4. To put the results in a more simple form: the greater the slope (1.72 in contrast to 0.86), the more rapid the recovery from the applied 3-indole acetic acid. These results may be related to the earlier ones in the following way: the earlier the flowering time, the slower the rate of root growth, and the later the time at which maximum root inhibition (and consequently recovery) occurs when the roots are treated with 3-indole acetic acid. The fact that a straight line results when the data are plotted as indicated in figure 4 appears to offer a simple relationship between root inhibition and concentration of applied growth substance. This relationship may be simply expressed as follows: time (in hours after germination to attain maximum inhibition)  $\div$  log. concentration (in mg./l.) = K, where K is the slope of the line.

Such a method of plotting appears to rule out the undesirable elements in the methods previously used (I.ane, 1936; Marmer, 1937). Here, the inhibitory effects of applied 3-indole acetic acid were ascertained by determining the percentage of inhibition as compared with the untreated control at the end of a given time. When percentage of inhibition is plotted against the logarithm of the concentration, one obtains a relationship which is difficult to express in simple mathematical terms.

Thimann and Lane (1938) have shown that the effect of applied growth substance on root inhibition wears off after the seedlings have been transferred to water. Some of our early experiments clearly indicated that the effect of the growth substance falls off, whether or not the seedlings are transferred to water. Thus, when measurements of root length are made at one time, some roots may have already been maximally inhibited and others not. From this point of view, this method fails to evaluate comparable factors. In the present method, one is measuring in all cases apparently comparable factors, namely, the

times required for maximal inhibition. The K value computed on the basis of such data seems to be a valid criterion of sensitivity to applied growth substance. The disadvantage of this method consists in the need for a series of measurements rather than one set of measurements in order to derive the growth curves from which the necessary data are computed

It has been shown that the two varieties here studied differ in rate of early shoot growth and number of roots at the end of a certain time, as well as in rate of early root growth and flowering time. An initial higher auxin concentration with continued high auxin production in a variety like Fulghum might very well account, at least in part, for all the growth correlations cited above. Preliminary auxin determinations made by the authors on dry seeds, however, show that the initial auxin concentration of Black Norway is higher than that of Fulghum. Such an auxin differential might very well account for the fact that the root and shoot emerge first in Black Norway as compared to Fulghum. The faster rate of shoot growth, and the relatively slower rate of root growth, as well as the greater number of roots with time as exhibited by Fulghum can be reconciled only with the assumption that this variety produces auxin at a faster rate following germination. This assumption is strengthened by the following consideration. It has been shown (Skoog, 1937) that when young oat seedlings are deseeded, they become more sensitive to applied growth substance. This increase in sensitivity is believed to be due to a lack of auxin brought about by the removal of the seed. One would expect, therefore, that if a coleoptile of a plant normally possesses less auxin than another, it would be more sensitive to applied growth substance. In our experiments, Black Norway shows a greater sensitivity in the Avena test to 3-indole acetic acid applied in agar blocks.

Another group of data further strengthens the assumption that Fulghum produces more auxin than Black Norway. When the roots are treated with 3-indole acetic acid, the shoots of Black Norway are stimulated while those of Fulghum are relatively unaffected and appear to be inhibited by high concentrations. This may indicate that Black Norway is deficient in growth substance, the addition of which stimulates the growth of the shoot. Fulghum, on the other hand, is "satiated" with growth substance (Greenfield, 1937), the addition of which produces no stimulating effect. Or to put the results in another way: for Fulghum, there is sufficient growth substance, further addition producing no increased effect, since factors other than growth substance now become limiting in growth. For Black Norway, growth substance is still limiting; addition of growth substance produces acceleration until some other factor becomes limiting.

At the same time that shoots are being stimulated by applied growth substance in Black Norway, roots are being inhibited. Root length in Fulghum is also being inhibited while high concentrations of growth substance are inhibiting shoot growth. All concentrations reported upon (0.002 mg./l. to 50.0 mg./l. for a variety like Black Norway, which is assumed to produce auxin at a slower rate) have been found to inhibit root growth. Concentrations between 0.00004 mg./l. and 0.0002 mg./l., on the other hand, have been found by the authors to be stimulating up to about 15 per cent as compared with the controls. For roots, therefore, exceedingly low concentrations of growth substances are stimulating, higher concentrations inhibiting. Those concentrations which stimulate root growth produce no effect on shoot growth. Stimulation begins to be evident for Black Norway at concentrations of about 0.002 mg./l. and continue to about 2.0 mg./l., after which further increases bring about inhibition. No such stimulatory effect is apparent in a variety like Fulghum.

With respect to number of roots, a higher rate of auxin production is again indicated in a variety like Fulghum since this variety possesses a larger number of roots at the end of 230 hours than Black Norway. For root number, however, auxin has not become limiting for both varieties, so that further addition of growth substance up to as much as 50.0

mg./l. continues to produce an increase.

All the above facts, therefore, point to a difference in auxin production between Fulghum and Black Norway. Furthermore, this difference is sufficient to account at least in part for all the correlations pointed out above with respect to root and shoot growth as well as root number. These results appear to support Thimann's (1937) view that plant parts respond to auxin in essentially the same way, except that they differ in their sensitivity to it.

Detailed experiments have already been undertaken to test the assumptions outlined above.

### SUMMARY

A simple method is described for carrying out growth measurements on oat seedlings over a period of four to five days.

Differences in early root and shoot growth (in the absence of light) are described for two varieties of Avena sativa. Black Norway, a late flowering variety, shows a more rapid root growth and a slower shoot growth than Fulghum, an early flowering variety.

The roots of these two varieties, when treated with aqueous solutions of 3-indole acetic acid ranging in concentration between 0.01 mg./l. and 5.0 mg./l., show the same maximum inhibition. The time at which maximum inhibition occurs is essentially the same in both varieties at low concentrations, but is later for Fulghum than for Black Norway at higher concentrations.

There is a direct relationship between the time at which maximum inhibition occurs and the logarithm of the concentration of 3-indole acetic acid used. The straight line plots have markedly different

slopes in the two varieties.

Concentrations of 3-indole acetic acid up to 50.0 mg./l. increase root number in both varieties in a similar manner. Low concentrations (0.002 mg./l. or less) are relatively ineffective in stimulating root formation, the effect being more pronounced with higher concentrations.

The two varieties differ in the effect on early shoot growth of 3-indole acetic acid applied in solution through the roots. Black Norway shoots show more rapid growth when treated with concentrations between 0.002 mg./l. and 2.0 mg./l., while Fulghum shoots remain unaffected. In addition, the coleoptiles respond differently in the *Avena* test, Black Norway appearing to be more sensitive.

The above results are interpreted in part on the basis of an assumption that Fulghum normally produces auxin at a faster rate than Black Norway.

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# THE DAILY CYCLE OF APPARENT PHOTOSYNTHESIS IN A SUBMERGED AQUATIC $^{\scriptscriptstyle 1}$

Bernard S. Meyer

The daily cycle of apparent photosynthesis in terrestrial species, under conditions approximating those which such plants encounter in nature, has been studied by Kostychev et al. (1926, 1928), Beljakoff (1928), Thomas and Hill (1937), and others. Such investigations have contributed to an understanding of the fundamental physiology and the ecological behavior of a number of species of plants. The results of two such investigations upon submerged vascular plants are also recorded in the literature.

Kostychev and Soldatenkow (1926) studied the daily variations in the rate of photosynthesis in a number of species of algae and submerged vascular aquatics. Apparent photosynthesis was measured in terms of oxygen evolution, which was determined by the Winkler method. In general their results seemed to indicate attainment of a maximum rate of photosynthesis in the mid-portion of the day, but most frequently before noon, and a usually consistent decrease in rate thereafter. The frequently found skewing of the curves toward the morning hours was attributed to the limiting effect of some internal factor, probably the accumulation of the products of photosynthesis.

A paper by Gessner (1938), part of which is devoted to a consideration of the daily cycle of photosynthesis in a number of species of submerged vascular aquatics, appeared after the experimental portion of this investigation was completed. Gessner employed a method which, in general, was similar to that used by Kostychev and Soldatenkow, except for modifications which eliminated or greatly reduced certain experimental errors present in their work. Plants were enclosed in 600 ml. glass flasks in water of reduced and known oxygen content and exposed to light while held in a horizontal position just under the surface of the water in a large pool. At the end of each hour, or in some experiments each half hour, the plant was transferred to another flask full of the water of low oxygen content, and the water from which the plant had been removed titrated for oxygen content by the Winkler method. Apparent photosynthesis was expressed in terms of oxygen evolution per hour. In general, Gessner found the daily curve of photosynthesis in submerged vascular aquatics to be closely correlated with the daily curve of radiation intensity.

The object of the investigation reported in this paper was the determination of the daily cycle of apparent photosynthesis in a representative submerged vascular aquatic species—Ceratophyllum demersum L.—under the most favorable light, bicarbonate concentration, and temperature conditions likely to be

1 Received for publication June 9, 1939.

Papers from the Franz Theodore Stone Laboratory and the Department of Botany, No. 410, of the Ohio State University. encountered by this species as it grows naturally in Lake Erie. Hence, only results obtained on clear days are reported. The experimental method employed was such that the rate of apparent photosynthesis could be determined for hourly intervals for a daily period of illumination without disturbing or handling the plant in any way.

EXPERIMENTAL PROCEDURE.—The apparatus employed incorporated some of the features of the experimental set-ups of Blackman and Smith (1911) and James (1928). In brief, the method consists in enclosing the plant to be studied in a glass chamber, circulating a solution of known and constant, but relatively low, oxygen content through this chamber, collecting the effluent stream of solution in a suitable pipette and determining its oxygen content. The rate of apparent photosynthesis<sup>2</sup> is calculated from the gain in the oxygen content of the water which has circulated through the plant chamber.

With the exception of the plant chamber, the water bath in which it was immersed, and the connecting tubes, the entire apparatus (fig. 1) was arranged on a table just inside a south-facing window of the laboratory. A tinned metal drum (A) of about 35 liters capacity, heavily coated with paraffin on the inside, was used as a reservoir for the affluent solution. The reservoir was provided with a round, snug-fitting float of paraffin about 1 cm. in thickness.

<sup>2</sup> The term "apparent photosynthesis" is used in the sense of photosynthesis minus respiration.

<sup>3</sup> The terms "effluent" and "affluent" are used with respect to the plant chamber.

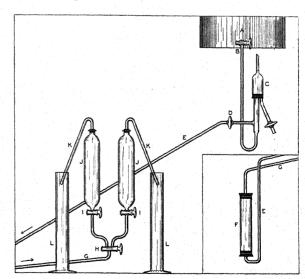


Fig. 1. Apparatus for the determination of photosynthesis in submerged water plants. A, reservoir; B, D, H, II, stopcocks; C, constant level device; E, G, glass tubes; F, plant chamber; IJ, pipettes; KK, overflow tubes; LL, graduated cylinders.

This served to prevent solution of atmospheric oxygen in the solution of low oxygen content in the reservoir. The reservoir was provided, on opposite sides at the bottom, with two glass stopcocks inserted through rubber stoppers. One of these (not shown in the figure) was used to obtain samples for testing the oxygen content of the solution in the reservoir. The other (B) was connected to a constant level device (C). From this device the affluent stream of solution passed through stopcock D and the downward slanting glass tube E out through the adjacent window to the plant chamber.

A glass cylinder, 4 cm. in inside diameter and 20 cm. in height was used as the plant chamber F (insert of fig. 1). This was fastened in position and immersed in the water contained in a large galvanized iron tub (diameter about 75 cm.; depth about 40 cm.). This tub was blackened on the inside and served as a water bath. It was supported on a low table a short distance outside the laboratory window. The temperature of the water bath was maintained nearly constant by a continuous flow of lake water which entered through a hose and escaped by overflowing the rim of the tub.

The glass cylinder which was employed as a plant chamber was stoppered, top and bottom, with tight-fitting rubber stoppers. The affluent stream of solution entered through the glass tube E which was tapped into the bottom stopper. The effluent stream left through the glass tube G which was inserted into the upper stopper. The effluent stream from the plant chamber passed through the glass tube G back into the laboratory to the three-way stopcock H which in turn was connected with the two cylindrical 700 ml. pipettes, J, J.

The passage of the stream of solution through the entire system is brought about by the hydrostatic pressure resulting from the difference in elevation of the top of the water column in the constant level device and the lower ends of the overflow tubes K, K. The arrangement of the apparatus is such that the circulating solution moves "uphill" from the bottom of the plant chamber to the top of the pipette. This facilitates clearing out any air bubbles which become entrained in the apparatus while it is being prepared for an experimental run. The stoppers at the top of the plant chamber and at the top of the pipettes were hollowed on the bottom in order to prevent air bubbles being entrained at those points when they were carried through the apparatus.

Although the hornwort is a rooted submerged aquatic, the apical portions of the plants, which are the most active photosynthetically, are commonly located just under the surface of the water. The apical portions of healthy, vigorous plants were collected directly from open water in shallow portions of Lake Erie the evening before a determination started and kept in a large jar of lake water overnight.

About one hour prior to the beginning of a determination approximately 16 liters of distilled water, with an oxygen content about half that of water in equilibrium with the partial pressure of oxygen in

the atmosphere at the same temperature, were introduced into the reservoir. Reduction in the oxygen content of the water was effected by boiling sufficient quantities in 6-liter Pyrex flasks and cooling to room temperature with the neck of the flask blocked with a snug-fitting float so that atmospheric oxygen did not have free access to the water. The water was boiled and cooled the day prior to running the experiment.

Sufficient sodium bicarbonate to bring the concentration of the final volume of solution to 0.02 per cent was dissolved in a small quantity of distilled water and quickly but thoroughly stirred into the water in the reservoir. The paraffin float was then placed in position on top of the solution.

The upper stopper of the plant chamber was then removed and the entire apparatus to the top of the plant chamber was filled with fresh solution. The apical 15 cm. (approximately) was cut from a suitable plant, a small glass weight tied to its basal end, and the plant inserted in the plant chamber, in which it assumed an approximately vertical position similar to its usual orientation in nature.

The upper stopper of the plant chamber was then inserted in position and both stoppers tightly wired in place. Since the top of the plant was about 5 cm. below the upper stopper, and since the tube was slanted slightly away from the direction of the noonday sun, the plant was at no time during the day in the shadow of the upper stopper.

The solution was then allowed to flow rapidly through the apparatus while connected to an empty pipette until the entire system was cleared of air bubbles. As soon as this was accomplished, flow was adjusted to the standard rate of about 800 ml. per hour. This rate of flow was maintained through the apparatus for at least half an hour before the beginning of the experimental run in order to allow the plant to attain a dynamic equilibrium with the solution.

Each daily series of determinations was begun at 5:30 or 6:30 a.m. At this time the effluent stream of solution from the plant chamber was switched through stopcock H to the other pipette, which had previously been filled with a solution of methylene blue in 10 per cent ethyl alcohol. The rate of solution flow was readjusted to 800 ml. per hour by proper manipulation of stopcocks B, D, and I.

The entering solution gradually displaced the methylene blue solution from the pipette through the overflow tube K into the glass graduate L. The rate of flow was checked at approximately 10-minute intervals, being followed closely by the rise in level of the liquid in the graduated glass cylinder. The exact flow was determined at the end of each hour by weighing the water which had been displaced into the graduate.

Because of its lower density the methylene blue solution floats as a distinct layer above the entering water. A sharp interface is maintained between the two layers of liquid. Interchange by mixing between the two liquids therefore does not occur, and any exchanges which occur by diffusion are probably small.

At the end of an hour all the methylene blue solution has been displaced from the pipette and about 100 ml. of the effluent solution has also overflowed into the graduated cylinder. At the end of each hour's run the effluent stream of solution is switched to the other pipette, which in the meantime has been filled with fresh methylene blue solution.

The purpose of the methylene blue solution was twofold: (1) to prevent direct contact of the effluent solution with the atmospheric oxygen and (2) to prevent a sudden change in the effective hydrostatic pressure which would ensue if the effluent solution were switched to an empty pipette at the end of each hour.

Duplicate 100 ml. aliquots of the solution in the pipette and of the solution in the reservoir were withdrawn at the end of each hour, suitable precautions being taken to avoid contact of the solutions with the atmosphere. The samples were analyzed for oxygen by the Winkler method, following with slight modifications the method as outlined in Theroux, et al. (1936). For a recent evaluation of this method for use in biological work see Allee and Oesting (1934).

The volume of oxygen liberated from the plant during each one-hour period was computed by multiplying the excess oxygen content of 100 ml. of solution from the pipette over that in 100 ml. of solution from the reservoir by the number of 100 ml. units (approximately 8) of water which entered the pipette during that hour. The rate of oxygen evolution was used as an index of the rate of apparent

photosynthesis.

The oxygen content of the solution in the reservoir usually showed a small rise during the day, probably due to a slight leakage at the edge of the paraffin float. This increase never amounted to more than 5 per cent of the amount originally present. For any given hourly interval, however, the oxygen content of the solution entering the plant chamber

was virtually constant.

As is well known, bubbles of gas containing a large proportion of oxygen often escape from submerged aquatic plants when illuminated. The more nearly static the water surrounding the plants and the more nearly saturated it is with respect to the partial pressure of oxygen in the intercellular spaces, the more likely gas bubbles are to escape from the plants. Obviously if any escape of oxygen occurred from the plants in gaseous form during these experiments, the results would be invalidated, as the method employed provides only for the analysis of dissolved oxygen. The procedure followed, however (that of immersing the plants in a continuously flowing stream of water of relatively low oxygen content) obviated this difficulty, and emission of bubbles did not occur at any time from the plants while they were being used in the experiments reported in this paper.

RESULTS AND DISCUSSION.—The results of four determinations of the daily cycle of apparent photosynthesis in *Ceratophyllum demersum* are shown graphically in figure 2. The number on each curve

corresponds to the day in August, 1937, on which the determination was made. For convenience these numbers will be used to refer to the individual curves.

As shown in this figure, the rate of apparent photosynthesis rose rapidly during the early morning hours to a peak value which was attained in the Au-

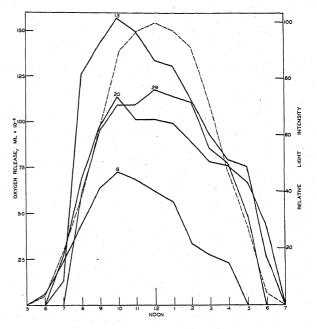


Fig. 2. Daily cycle of apparent photosynthesis in *Ceratophyllum demersum* on August 8, 13, 20, and 29, 1937 (solid lines). Daily variation in horizontal component of solar radiation on August 13, 1937 (broken line).

gust 29 curve at 12 noon, and in the other curves at 10:00 a.m. In general, after the peak rate had once been attained the rate of apparent photosynthesis declined consistently during the rest of the day, falling to a zero value by late afternoon. Actually, during the hours which are designated with a zero value for oxygen release, consumption of oxygen was occurring in the process of respiration.

In general the curves show a fairly close correlation with the daily cycle of solar radiation on a clear day, except that most of them are skewed toward the morning hours. This skewing is particularly marked in curves 8 and 13 and is also apparent in curve 20. Curve 29, on the other hand, is essentially symmetrical with a peak rate of apparent photosynthesis during the midday hour. Of four other similar determinations made during July and August, 1937, two give clear indications of a maximum of apparent photosynthesis before noon, and two give clear indications of a fairly symmetrical curve of apparent photosynthesis with the peak value at noon. The curves for these experiments are not reproduced because recognized experimental errors or temporary

<sup>4</sup> In figure 2 the figure 10 on the abscissa represents the hour from 9:30 to 10:30, etc. The time as given is true solar time.

periods of cloudiness invalidated one or more of the hourly determinations.

The possible rôle of each of the factors which may have influenced the daily cycle of photosynthesis in these experiments will now be considered.

Light.—The arrangement of the plant in the plant chamber was such that the radiant energy impinging upon it could not have differed materially from that received by similar plants growing at the same depth below the surface of the lake in clear, quiet water, under the same conditions of solar radiation. The quality and intensity of the light incident upon the plant were modified only slightly by its passage through the walls of the glass chamber, since the coefficient of light absorption for thin layers of glass is very low.

With certain minor exceptions, to be noted shortly, the plants were exposed to the daily cycle of solar radiation as it prevails on clear August days throughout each of the experiments. Hourly determinations of the horizontal component of solar radiation were made during each experiment with a Weston "Photronic" cell connected to a suitable milliammeter. Although such photovoltaic cells have definite limitations as instruments for measuring solar radiation, results obtained with them have at least a roughly quantitative significance. The curve of relative light intensity for August 13 is shown in figure 2. A subsequent checking of the photocell used against a calibrated pyrheliometer showed the peak value for solar radiation on this day to be about 1.25 g.-cal./cm.2/min., corresponding to an illumination value of about 8,500 foot candles. The daily cycles of intensity of solar radiation on August 8 and August 20 were closely similar to that for August 13, except that a severe thunderstorm greatly reduced the light intensity after 4:00 p.m. on August 8. On August 29 the intensity of solar radiation was slightly less (about 10-15 per cent) at all hours than on the other three days.

A correlation of the rate of apparent photosynthesis with light intensity is well marked in all the curves during the morning hours, and in some is also apparent for the afternoon hours. It is evident that the light intensity incident on a given leaf must attain a certain value if a maximum rate of photosynthesis is to be attained by that leaf under the other environmental factors prevailing. At light intensities above this value, light will no longer be the limiting factor in photosynthesis. This "optimum" light intensity is probably approximately the same for all the leaves on a given shoot. Hence, if all the leaves on a shoot are directly illuminated, an increase in the rate of photosynthesis should occur up to this "optimum," beyond which there will be no further increase with increase in light intensity. Such however, is not the situation. All the apical portions of the plants used consisted of one main branch to which were attached several shorter lateral branches. Because of this fact and the fact that the branches were densely clothed with leaves, some shading of interior by exterior leaves necessarily oc-

curred. Increase in light intensity will increase the incidence of light on all the leaves, but the exterior ones will be consistently exposed to a stronger light than the interior leaves. Hence, when the outer leaves are receiving light of "optimum" intensity, the incidence of light on inner shaded leaves may be much less.

It has been shown for a number of species of plants that the "optimum" light intensity for photosynthesis per leaf is less than the maximum possible solar illumination and this is undoubtedly also true of the hornwort. Because of the shaded condition of many interior leaves the rate of photosynthesis per shoot will increase with increase in the intensity of sunlight much beyond the optimum intensity for photosynthesis in a leaf which is fully exposed to radiation. The higher the intensity of solar radiation, the higher the average incidence of light on the leaves. The larger the proportion of the leaf surface which is sufficiently illuminated that light is no longer the limiting factor, the greater the rate of photosynthesis per shoot. The observed close correlation of photosynthetic rates with light intensity during all or part of the day probably can be largely ascribed to this effect. That shading of inner leaves by those at the periphery of the plant is an important factor in determining photosynthetic rates per plant in land species has been shown by Heinecke and Childers (1937), and it seems probable that this same effect also operates in submerged aquatics.

The possibility that the decrease in rate of apparent photosynthesis which was often observed in these experiments before the maximum of solar radiation was attained might be due to a retarding effect of high light intensities should be evaluated. According to Ruttner (1926), Schomer (1934), and others, when vascular aquatics are submerged at different depths in a body of water, the maximum rate of apparent photosynthesis often occurs at some distance below the surface rather than just under the surface. Similar results have been obtained by several investigators with algae. Since, however, both the quality and intensity of light change with its depth of penetration into water, the results of these experiments cannot be ascribed unequivocably to a retarding effect of high light intensities upon photosynthesis.

Arnold (1931) found the rate of photosynthesis of submerged aquatics to diminish with time when exposed to strong light under supposedly constant environmental conditions. This conclusion, however, is contradicted by the experimental work of Emerson (1935) and Gessner (1938). The results of the latter investigator indicate very convincingly that high light intensities (80,000–130,000 lux) per se do not exert a retarding effect upon photosynthesis in a number of species of submerged vascular aquatics including Ceratophyllum. Arnold's results, as is clearly pointed out by Gessner, can be ascribed to the use of a bubble-counting technique for measuring photosynthesis which is inherently unsuitable for use in such an investigation. It seems certain,

therefore, that the shape of the curves of apparent photosynthesis as obtained in this investigation cannot be accounted for by a retarding effect of high

light intensities upon photosynthesis.

Neither does it seem probable that high light intensities could exert an accelerating effect on respiration which would result in a diminution in the rate of apparent photosynthesis, since there is considerable evidence that light exerts no direct effect upon respiration (see, for example, McAlister, 1939).

Bicarbonate concentration of the water.—A 0.02 per cent solution of sodium bicarbonate (NaHCO<sub>3</sub>) was used in all experiments. The free carbon dioxide content of Lake Erie water, as would be expected from its pH value (approximately 8.0 in the summer), is negligible. The carbonate content of the waters of western Lake Erie, in terms of calcium carbonate, is about 90 parts per million during the summer months. Only a very small proportion of this (less than 5 per cent) represents carbonates, the remainder being in the form of bicarbonates. The calcium ion is present in Lake Erie water in a far greater concentration than any other cation, so there is no doubt that Ca(HCO<sub>3</sub>)<sub>2</sub> is the principal source of carbon dioxide for aquatic plants in this

Since one part by weight of NaHCO3 is equivalent to 1.19 parts of CaCO<sub>3</sub>, the bicarbonate concentration of the solution used was slightly more than twice as great as the usual values for western Lake Erie waters. This higher bicarbonate concentration was deliberately chosen because it was desired to have conditions in these experiments correspond to the most favorable conditions which ever prevail for photosynthesis in Lake Erie, and it is considered possible that higher bicarbonate concentrations than those reported above may sometimes exist in the lake.

It is obvious from the shape of the resulting curves that the bicarbonate concentration of the solution could not have been a limiting factor in photosynthesis for the entire shoot as a unit, except

possibly when peak values were obtained.

Oxygen content.—The oxygen content of the solutions entering the plant chamber was only about one-half that of saturated6 water at the same temperature. The water from the shallower portions of western Lake Erie is usually approximately saturated with oxygen. The oxygen content of the solutions used in these experiments was therefore considerably less than that encountered by submerged aquatics under natural conditions in this region. Actually the oxygen content of the water in the plant chamber often considerably exceeded that of the water entering from the reservoir due to the evolution of oxygen in photosynthesis.

<sup>5</sup> The figures quoted in this paragraph are based on

analyses by Mr. Leonard Bodenlos.

<sup>6</sup> In this paper the term "saturated" is used to describe solutions in which the oxygen content is in equilibrium with the partial pressure of the oxygen in the atmosphere at the same temperature.

There are no valid grounds for believing that variations in the oxygen content of the solutions of the magnitude which occured in these experiments have any appreciable effects upon the rates of either photosynthesis or respiration. Although there is some evidence that oxygen is necessary for photosynthesis, all available data indicate that only a very great reduction in the oxygen concentration of an air or water environment has any appreciable effect on this process. Likewise a diminution in the oxygen content of water has little effect on the rate of respiration unless it is very marked. Gessner (1937), for example, has shown for several submerged aquatic species that the oxygen content of the water must be reduced to 20 per cent of the saturation value or less before the rate of respiration is appreciably influenced.

Rate of flow of the solution.—The rate of flow through the plant chamber (volume about 200 ml.) in these experiments was 800  $\pm$  25 ml. per hour and was hence equivalent to approximately four complete changes of the water in the plant chamber per. hour. This rate was necessarily chosen somewhat arbitrarily, but equivalent natural agitation of the water is undoubtedly of frequent occurrence in Lake Erie. The rate of change of solution in the plant chamber was sufficiently rapid to obviate any appre-

ciable lag effect in the apparatus.

Temperature.—Since the plant chamber was immersed in a tub through which lake water was circulated rapidly, its temperature, as indicated by a thermometer inserted through the upper stopper (not shown in fig. 1), did not deviate greatly from that of the lake water. The temperature of the water in the plant chamber was  $26 \pm 1$  °C. in experiments 8, 13, and 29, and  $27 \pm 1$  °C. in experiment 20. These correspond approximately to the highest temperatures attained by Lake Erie water in the island region during the summer months. As a result of direct insolation effects on the water bath the temperature of the plant chamber was always slightly higher (1-2°C.) during the afternoon hours than during the morning hours.

It is evident from the shape of the curves in figure 2 that temperature could not have been the limiting factor in determining rates of photosynthesis in these experiments except possibly when peak rates were attained. Particularly it should be noted that the frequently observed skewing of the curves toward the morning hours cannot be ascribed to the temperature factor. It might be argued that the slightly higher afternoon temperatures induced a proportionately greater rise in the rate of respiration than in the rate of photosynthesis, thus causing a decrease in the rate of apparent photosynthesis. The fact that the rate of apparent photosynthesis often began to decrease by 10 o'clock in the morning renders such an explanation untenable.

Internal factors.—Since the frequently observed skewing of the curves of apparent photosynthesis cannot clearly be traced to any one or a combination of environmental factors, the possibility that this

effect may be due to the operation of some internal factor should be considered. One such factor is the accumulation of the products of the photosynthesis, which, according to Kostychev et al. (1926), Tschesnokov and Bazyrina (1930), Kurssanow (1933), and others, exerts a retarding effect upon photosynthesis in terrestrial species. Under certain conditions, as a result of the effect of this factor, the peak rate of photosynthesis may occur before noon.

Kostychev and Soldatenkow (1926) claim to have demonstrated such an effect of the accumulation of the products of photosynthesis in submerged aquatic species, but their experimental procedure is open to considerable criticism. Gessner (1938), on the other hand, considers that there is no basis for believing that the daily course of apparent photosynthesis in submerged aquatics is appreciably influenced by internal factors.

As far as the results of the present investigation go, at least a possibility that the daily course of photosynthesis is influenced by internal factors must be recognized. The fact that the frequently observed skewing of the daily curves of photosynthesis toward the morning hours cannot be traced to the influence of any environmental factor suggests that some internal factor—quite plausibly the accumulation of photosynthetic products—may often be exerting a limiting effect, especially during the afternoon hours. The existence of such a factor or factors, however, has not been experimentally confirmed.

#### SUMMARY

An apparatus is described with which it is possible to make consecutive hourly determinations of the rate of apparent photosynthesis in a submerged aquatic without disturbing or handling the plant in any way during the course of an experiment. On clear days in late July and in August with the temperature of the water in which the plant is immersed showing only a slight daily variation, the rate of apparent photosynthesis in apical shoots of Ceratophyllum demersum L. shows a rapid rise during the morning hours to a peak value which is attained between 10 a.m. and 12 noon, after which it shows a consistent decline. In general the daily curve for apparent photosynthesis shows a close correlation with the daily curve for the intensity of solar radiation, especially during the morning hours. Variations in light intensity are undoubtedly the principal factor involved in changes in the magnitude of apparent photosynthesis in this species from hour to hour throughout the day. The frequently observed skewing of the curves of apparent photosynthesis toward the morning hours, which apparently cannot be attributed to any environmental factor, suggests the possibility that an internal factor or factors may also exert an influence on the daily course of photosynthesis in this species.

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# CYTOPLASMIC INCLUSIONS IN THE MALE GAMETES OF LILIUM 1

Lewis E. Anderson

THE RESULTS of several investigations conducted during the past few years indicate that the male gametes in a number of angiosperms are complete cells. According to these workers, a distinct zone of cytoplasm surrounds the male nucleus during its passage down the pollen tube, and a few workers have described cellular male gametes in the embryo sac. The subsequent entrance of the male cell into the egg has not been observed, but it is conceivable that the entire cell may enter the egg. Thus, male cytoplasm would be introduced into the egg during fertilization. Little is known concerning the cytoplasm of male gametes, and no one has demonstrated the presence or absence of cytoplasmic inclusions. It seemed of interest, therefore, to study the gametes of Lilium, which have been shown to be cells by Cooper (1936), to see if they contain inclusions similar to those which have been described as occurring in the vegetative cells of the pollen tubes of many other plants.

Nicolosi-Roncati (1910), and more recently Wagner (1927), observed mitochondria during reductional division in *Helleborus foetidus* and found that they were about equally distributed among the resulting microspores. Similar observations have been made by Lewitsky (1911) on *Asparagus*, Bonnet (1912) on *Cobea*, Guilliermond (1912) on certain cucurbits and *Lilium* (1924), Wóycicki (1923) on *Malva*, Noack (1921) on *Pelargonium*, Dangeard (1922) on *Iris*, and Anderson (1936) on *Hyacin*-

thus.

Plastids and mitochondria are generally known to be almost universally present in the vegetative cell of pollen grains of angiosperms. In some plants the elaboration of starch by plastids in the pollen grains is assumed to furnish food for the growth of the pollen tube. Less is known, however, concerning the cytoplasm of the generative cell. Ruhland and Wetzel (1924) described plastids in recently formed generative cells of Lupinus, but they found that the plastids gradually disappear as the generative cell passes down the pollen tube. These authors asserted that chondriosomal bodies in the older generative cells originate from the evanescent plastids. Guilliermond (1924), on the other hand, was unable to demonstrate starch at any time in the generative cells of Lilium, but he found starch to be abundant in the vegetative cell. Anderson (1936) found abundant mitochondria and plastids in the pollen tubes of Hyacinthus and Antirrhinum and observed that

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these inclusions are especially aggregated around the male gametes. Nothing is known, however, concerning the inclusions in the gametes themselves.

MATERIAL AND METHODS.—Three species of Lilium were used in the present study—L. regale Wils., L. tigrinum Ker., and L. philippinense Baker. The plants were grown on the campus of the University of Wisconsin and in the greenhouse of Duke University. Pollinations were carried on during the summer of 1938 at the University of Wisconsin and during the winter of 1938-39 at Duke University.

Pellen tubes were obtained for study by germinating pollen both artificially and naturally. For natural germination, stigmas were pollinated and the stigma and style removed at intervals between 24 and 72 hours. In some instances the entire style and stigma was fixed for 12 to 24 hours, embedded in paraffin, sectioned longitudinally, and stained in Heidenhain's iron-alum haematoxylin. In others, the styles were slit longitudinally, the pollen tubes carefully removed, placed on a slide, fixed for one to two hours, stained, and mounted whole in canada balsam. Although satisfactory preparations were obtained by the latter method, it was necessary to handle the tubes with great care during the process of dehydration and staining to prevent them from twisting and stretching. When proper precautions were taken, however, tubes were obtained which compared favorably with those sectioned in the style. Cytoplasmic inclusions are more easily stained in pollen tubes which have been sectioned thinly, but they are unfavorable for study, since a section rarely extends very far through a given pollen tube.

The most satisfactory pollen tubes were obtained by germinating the pollen grains on slides which had been smeared with stigmatic exudate, according to the method described by Cooper (1936). The stigmatic exudate, which is produced freely in Lilium, was smeared on a chemically clean slide and the pollen dusted thereon. The slides were placed in a moist chamber and allowed to remain for 48 to 72 hours; they were then fixed for 1 to 2 hours, stained in iron-alum haematoxylin, and mounted whole in balsam. Some workers have criticized this method, stating that pollen tubes grown in this manner are abnormal, but no differences other than in size could be noted between pollen tubes grown in culture and those grown in the styles. Tubes grown in culture average considerably larger than those grown in the styles. This can be partially accounted for by the fact that those from the styles are shrunken somewhat because of the slow penetration of the fixing fluid.

A variety of fixing fluids were used. For stages where division figures were desired, several chromosmic mixtures were employed. The most satisfactory of these were Benda's, with and without acetic,

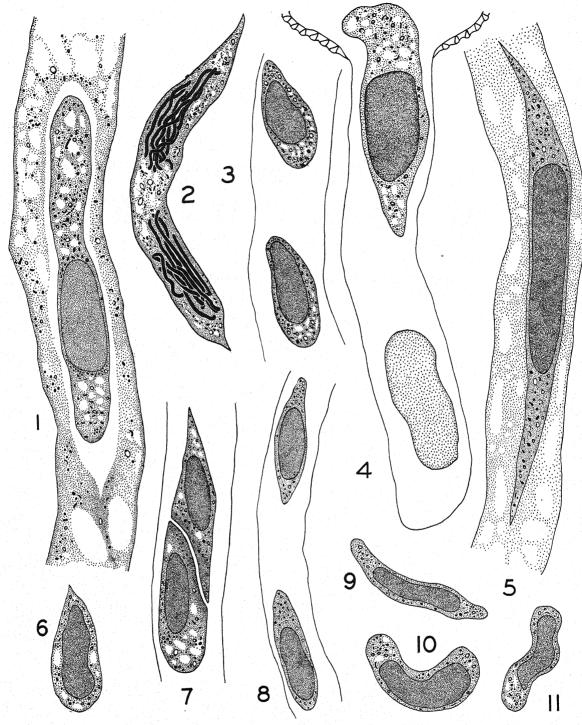


Fig. 1-11.—All figures were drawn with the aid of a camera lucida.—Fig. 1. Portion of pollen tube of *L. regale*, showing the generative cell. ×1280.—Fig. 2. Generative cell of *L. philippinense*, late anaphase. ×1020.—Fig. 3. Portion of pollen tube of *L. regale*, showing two male gametes shortly after their formation. ×1020.—Fig. 4. Tip of pollen tube of *L. tigrinum*, shortly after germination; the generative cell is just entering the pollen tube; tube nucleus shown at the tip. ×1020.—Fig. 5. Portion of pollen tube of *L. regale*, showing the long and attenuated generative cell. ×1280.—Fig. 6. Male gamete of *L. regale*. ×1020.—Fig. 7. Portion of pollen tube of *L. regale*, showing two male gametes before separation. ×880.—Fig. 8. Portion of pollen tube of *L. tigrinum*, showing two male gametes. ×1020.—Fig. 9 and 10. Male gametes of *L. philippinense*. ×1020.—Fig. 11. Male gamete of *L. regale*. ×1020.

Bensley's, and Regaud's fixatives. These gave good results with pollen tubes which had been grown on slides and were as a consequence directly exposed to the action of the fixing fluid. Since they penetrate very slowly, these fixatives were less satisfactory when entire styles were fixed. Where division figures were not desired, Zirkle's modification of Erliki's fixative (without pyridine) was used. This fixative produces a typical basic image. The chromatin is dissolved, and the nucleus is preserved as a solid mass of precipitated nuclear lymph. All figures, with the exception of figure 2, were drawn from pollen tubes fixed in this fluid. Figure 2 was drawn from material which had been fixed in Benda's fluid.

OBSERVATIONS.—The confusion of terms used to designate cytoplasmic inclusions necessitates a definition of those employed in the present paper. Most cytologists agree that plastids originate from visible primordia in the cytoplasm. Some maintain that plastid primordia and mitochondria are separate and distinct bodies, but thus far attempts to distinguish between them by means of stains, size, shape, etc., have failed. Sorokin (1938) reported that Janus Green B stains mitochondria when exact control of pH is maintained, but not plastid primordia. Attempts of the writer, however, to distinguish between plastid primordia and mitochondria in pollen tubes of Lilium with the use of Janus Green B were unsuccessful. Some inclusions are colored by the dve and others are not, as Sorokin observed, but there is no evidence that the unstained inclusions are the primordia of plastids. The inclusions which stain with Janus Green B are morphologically similar to those which do not stain, and it has not been demonstrated that Janus Green B shows any specificity for mitochondria in plants. For convenience, therefore, the term "mitochondria" will be used to include the primordia of plastids. The term plastid is reserved for those bodies containing starch.

The abundant inclusions in the pollen grains of Lilium are crowded in the cytoplasm between numerous and regularly arranged vacuoles. Guilliermond (1920) described a similar vacuolar system in L. candidum, and Anderson (1936) found the same condition in certain other plants with large pollen grains. Although the vegetative cell of the pollen grain is almost filled with oil globules, numerous mitochondria and plastids occur as well. These have been adequately described in vegetative cells of other plants (Guilliermond, 1924; Anderson, 1936).

The generative cell in ungerminated pollen grains is a complete cell, bounded by a distinct membrane which separates its cytoplasm from that of the vegetative cell. Starch is lacking at the time the pollen is mature, although Guilliermond (l.c.) described starch and plastids in the generative cell during earlier stages. Mitochondria are present in abundance throughout the cytoplasm, but they are especially aggregated around the elongated nucleus. Mitochondria are in the form of solid spheres and rods. A few are thread-like, and many are variously curved.

Many hollow spheres occur, as well as rods which are vacuolate at one or both ends. There is no evidence of plastids in the generative cell at any stage of its development. Guilliermond (1924) found starch in the generative cells of *L. candidum* immediately after they are formed, but concluded that starch, and consequently the plastids, soon disappears following pollination.

Shortly after the pollen grain germinates, the generative cell and tube nucleus pass into the pollen tube. The tube nucleus usually precedes the generative cell (fig. 4), but sometimes the generative cell enters first. If the latter occurs, the tube nucleus passes the generative cell after a short time. The tube nucleus varies in shape, size, and staining reaction. In most instances it is considerably larger than the generative nucleus and is globular or pearshaped (fig. 4). Sometimes, however, it is elongated, appearing almost thread-like. This appearance may be caused by poor fixation or by the beginning of its disintegration. The tube nucleus is always free in the cytoplasm of the pollen tube, and frequently cytoplasmic inclusions are massed around its periphery.

The cytoplasm of the generative cell is bound by a distinct membrane, which separates it from the cytoplasm of the vegetative cell (fig. 1, 2, 5). Although the nature of this membrane was not studied, its presence can be easily demonstrated in fixed material. The membrane is particularly evident when there is excessive shrinkage following fixation and dehydration, for then the generative cell is completely separated from the cytoplasm of the pollen tube (fig. 1). Most preparations show this space around the generative cell to a greater or lesser extent, but in some preparations it is absent (fig. 5). There is no evidence that the membrane is other than a cytoplasmic one.

Vacuoles are present in the cytoplasm of some generative cells and almost entirely absent in others. Cells which are exceedingly long and attenuated lack vacuoles (fig. 5). Generative cells, on the other hand, which are short and thick usually exhibit numerous vacuoles scattered throughout their cytoplasm (fig. 1). Mitochondria are exceedingly abundant. They are preserved in the form of solid and hollow spheres, straight and curved rods, and chains. Plastids are altogether lacking, although the characteristic stages, intermediate between mitochondria and plastids, are present (fig. 1, 5).

The division of the generative cell occurs 48 to 72 hours after pollen germination. Nuclear division has been described in detail by Cooper (1936), who reported the division as normal in every respect. The prophases are of long duration, the spireme being initiated shortly after the pollen tube is formed. During the prophase mitochondria are grouped abundantly around the nuclear membrane, and when the nuclear membrane disappears just prior to the formation of the equatorial plate, inclusions are scattered throughout the region of the spindle. Spindle fibers were not observed, but Cooper (1936) de-

scribed a typical spindle in *L. regale*, *L. auratum*, and *L. philippinense*. The chromosomes at metaphase are exceedingly long, and after the halves separate, a single chromosome may extend for half the length of the generative cell. The separation of the chromosomes and their movement to the poles during anaphase is normal (fig. 2). In some generative cells, which have become exceedingly long and narrow, the chromosomes are crowded together and occupy almost the entire central region of the cell. Mitochondria and other inclusions in such cells are mostly confined to the extremities, but they may be so numerous that they clump together in a solid, dark-staining mass which may completely occupy the attenuated ends of the generative cell.

After the chromosomes separate at metaphase and pass to the poles, mitochondria are often present in the region of the equatorial plate (fig. 2). Cell plate formation, and the subsequent division of the cytoplasm to form the two male gametes, evidently takes place rapidly. The details of the formation of the cell plate and the formation of the two gametes, were not followed, for Cooper (1936) described this process in detail. When the cytoplasm divides, inclusions are incorporated into each of the resulting

male gametes (fig. 7).

The male gametes are complete cells (fig. 3, 7) as soon as they are formed, having derived their cytoplasm and inclusions from the generative cell. The two gametes soon separate (fig. 3), and each contains abundant mitochondria. There are no connecting strands of cytoplasm between the gametes as have been reported in Vallisneria (Wylie, 1923) and Oenothera (Ishikawa, 1918). Each gamete is completely independent of the other. The gametes remain independent throughout the course of their passage down the pollen tube, and each retains its surrounding layer of cytoplasm (fig. 8). Some male cells are vacuolate especially at the end toward the direction of movement (fig. 3, 6, 11), while others show no evidence of vacuoles at all (fig. 8). The inclusions in the gametes are similar to those in the generative cell. Mitochondria are abundant, existing as solid-staining spheres, straight and curved rods, hollow spheres, and threads. Tests for starch failed to reveal the presence of any plastids.

The male gametes were found to persist as cells in all the material examined, and there is no indication that the layer of cytoplasm around the male nuclei disappears prior to their dischargal into the embryo sac. The pollen tubes were followed until they reached the base of the style, or in those artificially germinated, for a period of about 72 hours, and mitochondria were evident in all of these. Figure 8 was drawn from a pollen tube which had penetrated the style nearly to the cavity of the ovary. The male cells are distinct, and the inclusions are well preserved. These observations agree with those of Cooper (1936), who likewise found the gametes persisting as cells after they entered the embryo sac. Although Cooper did not observe inclusions, there is no reason to suppose they disappear before the gametes enter the embryo sac, as they are present in such abundance just prior to this (fig. 6-11).

Discussion.—Different observations have been made concerning the nature of the male gametes in Lilium. As early as 1891, Guignard figured the male gametes of L. Martagon as cells. Aside from the fact that Guignard pictured astral bodies in the gametes. his figures agree in all essentials with those in the present paper. Koernicke (1906) and Strasburger (1908) properly denied the existence of centrasomes, but they also denied the existence of the male gametes as cells. Strasburger described the male nuclei as vermiform, which, he suggested, results from a type of amoeboid movement. This view was supported later by Nawaschin (1910) and Blackman and Welsford (1913). Welsford (1914) found sperm cells in the pollen tubes of L. auratum and L. Martagon, but she claimed that the cytoplasm of the sperm cells disappears before the pollen tube reaches the ovary. Somewhat similar behavior was described by O'Mara (1933) in L. regale and L. auratum, in which he reported that the cytoplasm of the sperm cells gradually dissolves, leaving the nuclei naked in the cytoplasm of the pollen tube. More recently, however, Cooper (1936), working with L. Martagon, L. auratum, and L. philippinense, saw no evidence that the male gametes lose their cytoplasm, but to the contrary observed them as cells until they reached the embryo sac. The failure of other workers to observe the cellular nature of the male gametes is probably partially due to their use of staining techniques adapted for nuclear study which do not show cytoplasm to the best advantage. Pollen tubes when destained sufficiently to show nuclear structure plainly are too faint to show the outline of the zone of cytoplasm around the gametes.

Observations on other plants with respect to the nature of the male gametes differ as widely as do those on Lilium. Recent work seems to indicate, however, that the male gametes exist as cells in many more plants than has formerly been supposed. Wulff and Maheshwari (1938), after a thorough review of the literature dealing with the male gametophytes of angiosperms, predict almost universal occurrence of sperm cells. These writers cite numerous plants formerly reported to have naked male gametes which in later investigations proved to be complete cells.

The present studies not only support the work of these investigators, but indicate further that the cytoplasm of the male gametes possesses all the inclusions which are normally present in the vegetative cells of pollen grains. The presence of vacuoles also indicates that they are organized cells. Although plastids were not observed in the cytoplasm of the male cells, mitochondria are abundant, as are transition stages in the development of plastids from mitochondria. There is no indication that these inclusions disappear during the migration of the male gametes down the pollen tube (cf. fig. 7, 3), and it is logical to suppose that the gametes retain these inclusions as long as they remain cells.

Sperm cells have been observed in the embryo sacs of several plants. Nawaschin and Finn (1913) figured the male gametes as cells in Juglans regia and J. nigra immediately after the gametes are discharged into the embryo sac. Their drawings of later stages, however, do not show the gametes as cells, but as naked nuclei with a clear space around each. Finn, in a later paper (1925), states that this clear space was intended to represent a layer of cytoplasm and maintains that the male gametes in Juglans remain as cells until fertilization. Tchernoyarow (1915), in Myosurus minimus, and Ishikawa (1918), in Oenothera, figured the male cells after they had reached the embryo sac. Both observers claimed, however, that the male cytoplasm merges with the cytoplasm of the embryo sac, leaving naked male nuclei free in the embryo sac.

Although it has not been demonstrated that the male gamete enters the egg as a complete cell, many workers disagree with the idea of a disintegration of male cytoplasm while the gamete is yet in the embryo sac. Wylie (1923) in Vallisnaria spiralis found that the male gamete remains a cell at least until it reaches the surface of the egg. Although he was unable to follow the entrance of the male gamete into the egg, Wylie thinks it highly probable that some or all of the male cytoplasm accompanies the male nucleus into the egg. Finn's (1925) observations on Asclepias Cornuti are similar to those of Wylie, and Cooper (1936) observed that the male gametes of Lilium are still complete cells when liberated into the embryo sac. In Antirrhinum majus there also is some evidence that male cytoplasm enters the egg with the male gamete (Anderson, 1936).

If the male gamete enters the egg as a complete cell, then it is obvious that male cytoplasm would be introduced during fertilization. Since it has been shown that the cytoplasm of the male gamete contains numerous mitochondria and consequently the primordia of plastids, these would be introduced into the egg. Detailed studies of the exact mode of entrance of the male gamete are needed to determine whether the entire male cell enters the egg or whether the nucleus only participates in fertilization.

#### SUMMARY

Cytoplasmic inclusions were studied in vegetative and generative cells and in the male gametes of Lilium regale Wils., L. tigrinum Ker., and L. philippinense Bak. Pollen grains were germinated both artificially and on the stigmas. Mitochondria are present both in the vegetative cell and in the generative cell of the pollen grains. When the generative cell divides, mitochondria are distributed about equally to the two male gametes. The male gametes are complete cells, consisting of a nucleus surrounded by a distinct zone of cytoplasm which is sharply delimited from the cytoplasm of the vegetative cell. The male cells contain mitochondria and transition stages in the development of plastids from mitochondria. These inclusions are derived from the cytoplasm of the generative cell. The male gametes remain distinct cells at least as long as they remain in the pollen tube, and the cytoplasmic inclusions also retain their identity. These inclusions could, therefore, be transmitted to the egg if the entire male cell participates in fertilization.

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# RESPIRATION AND FERMENTATION IN THE CARROT, DAUCUS CAROTA. II. FERMENTATION AND THE PASTEUR EFFECT<sup>1</sup>

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In the preceding paper Marsh and Goddard (1939) presented results obtained with respiratory enzyme poisons showing that in carrot roots and in young carrot leaves a large fraction of the respiration is catalyzed by an oxidase similar to or identical with the cytochrome oxidase; in mature leaves a shift occurs in the oxidase to a system insensitive to cyanide, azide or carbon monoxide. The present paper is a study of the relation between respiration and fermentation in carrot root tissue. It will be shown that inhibition of respiration by lowered oxygen pressure, by cyanide, azide, or carbon monoxide results in increased fermentation.

It has long been known that many higher plants liberate  $\mathrm{CO}_2$  when placed in pure  $\mathrm{N}_2$  or other inert gas. Alcohol is also produced in many cases (Kostychev, 1931; Stiles and Leach, 1932). The ratio alcohol/ $\mathrm{CO}_2$  produced has been investigated for many plants; considerable variation in this ratio from one plant to another has been found, the values ranging from 0 to 0.99 (Kostychev, 1931). Although there has been much speculation as to probable importance of anaerobic  $\mathrm{CO}_2$  production in higher plants, very little research has been conducted in the field since

the later years of the nineteenth century.

It is well known that in yeast (Meyerhof, 1926), animal tissues (Warburg, 1930), and in higher plants (Kostychev, 1931; Blackman, 1928) either no fermentation occurs in air or it occurs at a lower rate than under anaerobic conditions. If lactic acid production (in animal tissues) in N2 and in air, and oxygen consumption in air, be converted into terms of sugar decomposition according to the equations (1)  $C_6H_{12}O_6 \rightarrow 2CH_3CHOHCOOH$  and (2)  $C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$ , it is found in many cases that more sugar is decomposed per unit time in N2 than in air. This phenomenon of an increased rate of sugar decomposition in transferring tissues from air to N2 is known as the Pasteur effect. The literature of the Pasteur effect has been well reviewed by Dixon (1937), Turner (1937), and Burk (1937). Although the transfer of a tissue from nitrogen into air allows oxidative sugar decomposition to occur (equation 2), at the same time there is such a large decrease in the rate of sugar decomposed by glycolysis (equation 1) that the result is an overall decrease in the rate of sugar decomposition. The ratio mols lactic acid in N2- mols lactic acid in air/mols O<sub>2</sub> in respiration, is known as the Meyerhof quotient (1926). Values of this quotient lie between 1 and 2 for many animal tissues (Burk, 1937) and for yeast (Meyerhof, 1926). (In the quotient for yeast, the term "mols CO2 of fermentation" is substituted for "mols lactic acid." The expressions <sup>1</sup> Received for publication June 30, 1939.

are entirely comparable since each mol of glucose produces two mols of CO2 of fermentation just as it produces two mols of lactic acid.) If we convert the Meyerhof quotient into terms of sugar decomposition by multiplying by 3, we obtain the ratio: No. molecules glucose fermented in N2- no. fermented in air/no. glucose molecules respired. This quotient then has values between 3 and 6. Meyerhof believes that respiration has an inhibitory effect on fermentation and interprets these figures as meaning that the respiration of one molecule of glucose inhibits the fermentation of three to six molecules of glucose. However, the Meyerhof quotient is essentially merely an expression of experimentally determined facts and a criterion for the existence and magnitude of the Pasteur effect; it does not necessarily support Meyerhof's oxidative resynthesis theory or any other particular explanation of the mechanism of the phenomenon.

The only detailed explanation of the Pasteur effect which has received wide acceptance is Meyerhof's oxidative resynthesis theory. This theory states that lactic acid, the end product of anaerobic carbohydrate breakdown in muscle, undergoes in part oxidation to  $\rm CO_2$  and water and in part resynthesis to carbohydrates in air, the energy from the oxidation being utilized in the resynthesis. The experimental evidence for the theory is collected in Meyerhof (1930).

The possibility that ethyl alcohol may be in part oxidized and in part resynthesized to carbohydrate in air was suggested as early as 1879 by Wortmann (quoted by Turner, 1937). One serious objection to Wortmann's proposal is that it will not account for a lower total CO<sub>2</sub> production in air than in N<sub>2</sub> such as is observed, for example, in Meyerhof's (1926) experiments with yeast. Meyerhof (1926) has suggested that in yeast a 3-carbon intermediate undergoes oxidative resynthesis to carbohydrate. Blackman (1928) has advanced a theory that in air a 3-carbon fermentation intermediate is in part oxidized and in part resynthesized to carbohydrate, a scheme not essentially different from that proposed by Meyerhof (1926) for yeast.

The Meyerhof lactic acid resynthesis theory rests upon a rather moderate, perhaps inadequate body of experimental evidence. The validity of several assumptions involved has been called into question (Burk, 1937; Dixon, 1935). Even the fundamental assumption that the Pasteur effect is due to some effect of respiration on fermentation has been considered invalid; Lipmann (1933) has observed that the glycolysis of a muscle extract is inhibited by several auto-oxidizable dyestuffs in the presence of air, but not in N<sub>2</sub>. He considers this experiment to be a

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model of the Pasteur effect, in which it is supposed that oxygen acts through some natural carrier or carriers with a fermentation enzyme, thereby inactivating it. The experimental basis of Lipmann's theory has been questioned by Michaelis and Smythe (1936).

MATERIALS AND METHODS.—Since the same general procedures and the same Fenn (1928) microrespirometer were used in this part of our study as in Part I, only those features of the methods which differed from those described in Part I (Marsh and Goddard, 1939) will be presented here.

In determining the rate of fermentation, the vessels were made anaerobic with tank nitrogen which had been freed of oxygen by passing over hot copper. A separate correction factor for the solubility of carbon dioxide was applied for each vessel. This factor was obtained from the equation:  $K_{CO_2} = V.C.$  [1 +  $\sigma$  (V<sub>1</sub>/Vg)].  $\sigma$  = absorption coefficient of  $CO_2$  in the medium;  $\sigma$  at 22° = 0.837;  $V_g$  = volume of gas space in the experimental vessel;  $V_1$  = volume of liquid in the experimental vessel; V.C. = vessel constant calculated according to Fenn (1928).

The R.Q. was determined on the same tissue, by measuring first the oxygen consumption at 10-minute intervals for one hour with CO<sub>2</sub> being absorbed by the KOH of the insets. The KOH was then removed by washing and the inset filled with an acid buffer containing a pH indicator. The movement of the bubble was again observed at 10-minute intervals for an hour. The CO<sub>2</sub> produced during the second period was calculated from the following relations:  $X_{CO_2} = X_{O_2} - X_{obs.}$ ; vol.  $CO_2 = X_{CO_2} K_{CO_2}$ ; vol.  $CO_2 = X_{O_2} K_{O_2}$ ; R.Q. = vol.  $CO_2/\text{vol.} O_2$ ;  $X_{obs.} = \text{linear movement of the bubble during second hour, where <math>X_{obs.}$  is positive if in the direction of the oxygen movement, negative if in the opposite direction;  $X_{O_2} = \text{linear movement}$  of the bubble during the first hour.

The validity of this method depends upon the constancy of the  $O_2$  and  $CO_2$  production over a considerable period (at least two hours). This was shown to be the case.

Ethyl isocyanide was prepared according to the method of Gautier (1869) and traces of HCN removed by the purification method of Toda (1926).

EXPERIMENTAL RESULTS.—Demonstration of the Pasteur effect.—Since one molecule of  $CO_2$  produced in fermentation represents three times as much glucose as one molecule of  $CO_2$  produced in respiration, if the quotient  $CO_2$  of fermentation/ $CO_2$  of respiration is greater than 1/3, the Pasteur effect is in operation. This is true only if the ratio  $C_2H_5OH/CO_2$  is close to 1 in accordance with the equation of alcoholic fermentation:  $C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$ . Kostychev (1931) gives values of this ratio for carrot root of 100/102, 100/91, 100/86, and 100/72. However, the Pasteur effect is shown to be in operation regardless of the alcohol- $CO_2$  ratio if the fermentation  $CO_2$ /respiration  $CO_2$  ratio is more than 1.

Although a large number of figures from control vessels of other experiments made it obvious that the ratio of anaerobic to aerobic CO<sub>2</sub> was above 1/3, a separate experiment was run to determine this ratio more accurately. Two vessels were run anaerobically and three in air; the results are presented in table 1.

Table 1. The ratio anaerobic CO2/aerobic CO2.

Gas exchange in mm.3/gm./hr.							
Total CO <sub>2</sub> in air	$\mathrm{CO}_2$ in $\mathrm{N}_2$						
87 (106) <sup>a</sup>	98.1						
92.6 (113)	111						
94.3 (115)	•••						
Average 91.3	104.5						

 $^{n}$  Figures in parentheses are the oxygen consumption figures, actually experimentally determined;  $CO_{2}$  in air was calculated by multiplying these values by an R.Q. of 0.82, determined the following day.

The R.Q. was determined the following day on carrots from the same source and found to be 0.82.

The carrot root slices were tested for production of organic acids in N<sub>2</sub> by measuring the CO<sub>2</sub> output when suspended in phosphate buffer and when suspended in M/100 KHCO<sub>3</sub> in equilibrium with 98 per cent N<sub>2</sub> and 2 per cent CO<sub>2</sub>. Acids stronger than carbonic would drive CO<sub>2</sub> from the KHCO<sub>3</sub> solution and result in a higher total evolution of CO<sub>2</sub> in these vessels. Two vessels with phosphate buffer yielded an average of 63.4 mm.<sup>3</sup>/gm./hr., while two vessels in the bicarbonate medium yielded an average of 67.0 mm.<sup>3</sup>/gm./hr. This slight difference is probably entirely experimental error, and no acid is produced.

Fermentation at low oxygen pressures.—Experiments with low oxygen pressures indicated that when considerable inhibition of respiration was caused by lowering the oxygen pressure, fermentation occurred, and the R.Q.2 consequently increased. Results of these experiments are shown in table 2. The determinations with 1 and 2 per cent oxygen were not of the highest accuracy; the rates of gas exchange were not very constant with time, this apparently being due to the decrease in oxygen pressure caused by respiration. Another difficulty arises in any experiment with tissue where the oxygen tension is limiting the respiratory rate; the oxygen tension may be appreciably lower in the center of the tissue than at the outer edges, and the rate of fermentation may not therefore be uniform in all cells. These criticisms are probably not too serious, since we obtained results at low oxygen tensions which are in good agreement with those obtained in cyanide or azide, reported later in this paper, which are free from either of the two objections suggested here.

 $^2$  Since in fermentation CO<sub>2</sub> is produced without a corresponding oxygen consumption, the respiratory quotient or ratio of total CO<sub>2</sub> produced to O<sub>2</sub> consumed is higher when both processes are occurring simultaneously than in the case of respiration alone.

Table 2. The effect of low oxygen pressures on R.Q.

Percent-		The State of			Percent-
age oxy-	Controls	Exper	imental		age inhib.
gen	in air	$O_2$	$CO_2$	R.Q.	of resp.
5	76	53.0	43.5	0.82	30
5	76	56.8	43.8	0.77	25
$2\frac{1}{2}$	(76)a	45.0	56.3	1.25	41
2	(76)	40.5	52.0	1.28	46
2	(76)	37.8	45.8	1.21	50
2	94.5	28.3	49.0	1.73	70
2	(94.5)	28.3	55.6	1.96	70
1	93.2	15.6	52.0	3.3	83
1	(93.2)	14.2	49.5	3.5	85
1	(93.2)	19.2	66.5	3.3	79

<sup>&</sup>lt;sup>a</sup> The parentheses indicate the use of a single control vessel to calculate percentage inhibition of respiration of more than one sample.

The determinations were sufficiently accurate to indicate the general trend of fermentation, increasing as lower oxygen pressures were used and as respiratory inhibition increased. These results might, however, be interpreted according to Lipmann as an effect of oxygen acting upon a fermentation enzyme by some other pathway than through the respiratory chain. In view of the current debate upon this question it seemed desirable to try to determine which explanation is correct, Lipmann's or the earlier idea that respiration itself has a direct inhibitory effect on fermentation.

Aerobic fermentation induced by cyanide, azide, carbon monoxide.—At this point a convenient method of separating the respiration and fermentation mechanisms in the carrot root was found. It was dis-

Table 3. Insensitivity of carrot root fermentation to carbon monoxide, cyanide and azide.a

	Ferme	ntation	mm.3C	$\mathrm{O}_2/\mathrm{gm}$ .
Cor	ntrol			Poisoned
	Sa	me samj	ole 30 m	nin.
in	$N_2$			on CO
4	7.5			44.9
5	6.0			57.3
5	7.8			54.7
		50 min	. in N <sub>2</sub>	
Co	ntrol			with 10-3 M cyanideb
4	7.8			43.2
3	9.2			43.8
		70 min	. in $\mathrm{N}_2$	
Co	ntrol			with 10-3 M azideb
8	3.0			69.5
8	1.6			73.8

<sup>&</sup>lt;sup>a</sup> From Part I it is known that about 2/3 of carrot root respiration is inhibited by 95% CO and about 75% by  $10^{-3}$  M azide or cyanide.

covered that carrot root fermentation was unaffected by the respiratory poisons (Marsh and Goddard, 1939) cyanide and carbon monoxide, as is the case with yeast (Warburg, 1925, 1927), and was only slightly sensitive to low concentrations of the respiratory poison sodium azide. Table 3 presents figures on these results.

Table 4. R.Q. and aerobic fermentation in  $5 \times 10^{-4} M NaN_3$ .

	$O_2$	$ ext{Total} \\  ext{CO}_2 \\$	$\begin{array}{c} \text{Aerobic} \\ \text{ferm.} \\ \text{CO}_2 \end{array}$	R.Q.	Percentage inhib. of resp.
Controls					
pH 4.5	74.2	54	• • •	0.73	
pH 5.1	80.2	73.8		0.92	
pH 5.7	68.4	61.6		0.90	
Experimen- tal					
pH 4.5	13.0	60.6	49.9ª	4.7	83
pH 5.1	19.6	59.2	42.6	3.0	75
pH 5.7	19.8	61.6	44.8	3.7	71

<sup>&</sup>lt;sup>a</sup> Fermentation  $CO_2$  was calculated by multiplying  $O_2$  consumption  $\times$  0.85, the average R.Q. of the controls, and subtracting the resulting figure from the total  $CO_2$ .

Table 5. Reversibility of effect of cyanide and carbon monoxide on R.Q. and aerobic fermentation.

			$\mathrm{mm.}^{3}/\mathrm{g}$	m./hr.				
In 10 <sup>-3</sup> M cyanide				Sa	ıme afte	r washi	ng	
Aerobic Total ferm.					Total	Aerobio ferm.		
$O_2$	$CO_2$	$CO_2$	R.Q.	$O_2$	$CO_2$	$CO_2$	R.Q.	
14.5	33.2	20.9ª	2.29	76.6	67.8		0.88	
11.5	33.9	24.2	2.95	60.3	54.0		0.90	
16.4	34.3	20.3	2.09	66.6	57.1		0.86	
14.0	33.9	22.0	2.42	64.0	57.0		0.89	
w	ith 95%	CO, 5%	$O_2$					
		dark			Same in	the ligh	nt	
24.3	87.3	66.7	3.59	53.1 <sup>b</sup>	69.4	24.3	1.30	
20.5	60.3	42.9	2.99	62.3	78.2	25.2	1.25	

 $<sup>^{</sup>a}$  Fermentation CO<sub>2</sub> was calculated by multiplying O<sub>2</sub> consumption by 0.85, the average R.Q. of unpoisoned tissue (table 6), and subtracting the resulting figure from total CO<sub>2</sub>.

It was then discovered that cyanide, azide, and carbon monoxide all induced aerobic fermentation in the carrot root (table 4, 5, 6), and for cyanide and carbon monoxide it was shown that this effect is reversible (table 5). If Lipmann's explanation of the action of an oxidizing mechanism, other than respiration, on a fermentation enzyme is correct,

<sup>&</sup>lt;sup>b</sup> Similar tissues but not same sample.

<sup>&</sup>lt;sup>b</sup> In this experiment we obtained R.Q. in 95% N<sub>2</sub>, 5%  $O_2 = 50.3$  mm. $^3$ CO<sub>2</sub>/gm./hr.  $\div$  63.3 mm. $^3$ O<sub>2</sub>/gm./hr. = 0.79.

then we must assume that each of these poisons, in the concentrations used, inhibits the action of Lipmann's oxidizing chain as well as respiration. A further difficulty is that the inhibition of the oxidation of the fermentation system would have to be light reversible. Lipmann's theory is consistent with our results only if the inhibition of fermentation by oxygen is catalyzed by cytochrome oxidase. Ball (1939) has suggested that the ratio of oxidized cozymase/ reduced cozymase may be considerably different in aerobic and anaerobic cells. Cozymase + specific protein constitutes the enzyme reducing acetaldehyde to alcohol. Further, cozymase + other specific proteins constitute the dehydrogenases catalyzing the oxidation of pyruvic acid and triosephosphates, etc. Since cozymase is not autoxidizable, the ratio of oxidized/reduced cozymase will not be determined by oxygen pressure alone. It is probable that cozymase undergoes oxidation by means of the coenzyne factor (diaphorase) + unknown carriers, cytochrome, cytochrome oxidase. Our results are consistent with Ball's suggestion, but in no way substantiate it.

One cannot calculate a Meyerhof quotient for tissues which do not glycolyse or ferment in air, for in these cases it is possible that not all of the respiration is necessary to completely prevent aerobic fermentation. However, it is possible to calculate a term essentially equivalent to a Meyerhof quotient, by using the respiratory and fermentation rates at two different levels of cyanide poisoning at both of which aerobic fermentation occurs. Similar calculations may be made from the data at two oxygen pressures, both sufficiently low to give fermentation but at different rates. Let X represent a concentration of

cyanide at which fermentation occurs, and Y a higher concentration of cyanide, then:  $CO_2$  of ferm. in  $Y-CO_2$  of ferm. in  $X/O_2$  of resp. in  $X-O_2$  of resp. in Y= "M.Q."

Due to experimental variation, our values with cyanide are not accurate enough to make calculations over narrow ranges of cyanide concentrations. If, however, we let  $X = 1.0 \times 10^{-4}$  M cyanide and  $Y = 8 \times 10^{-4}$  M cyanide, then we obtain (using average figures from table 6) for the above quotient a value of 29.8-1.2/46.2-14.3 = 0.90.

If we calculate a similar ratio (likewise expressing the efficiency of respiration in inhibiting fermentation) by using the average fermentation and respiration values for  $10^{-4}$  M cyanide (table 6) and the fermentation value in 100 per cent  $N_2$  (table 1), we obtain a quotient of 104.5-1.2/46.2=2.24. If we make the calculation between  $8\times 10^{-4}$  cyanide and 100 per cent  $N_2$ , the ratio equals 104.5-29.8/14.3=5.22, indicating that the first few per cent of respiration are more efficient in inhibiting fermentation than are additional increments.

The general trend of the effect of respiration on fermentation may be seen clearly from figure 1; as respiration is inhibited beyond about 50 per cent, R.Q. rises from about 0.85 to about 3. It is, of course, highly probable that in nature the cells of the carrot root, particularly those near its center where oxygen diffusion would be a rate-limiting factor, do not respire nearly so fast as do the thin slices used in our experiments.

Inhibition of the Pasteur effect.—Warburg (1926) has shown that M/1000 ethyl isocyanide inhibited neither respiration nor fermentation of Jensen sarcoma but allowed fermentation (glycolysis)

Table 6. Rise in R.Q. as respiration is inhibited by cyanide.

			Gas ex	change i	n mm.3/gm./	hr.		
Molar con- centration	Time in min.	Mg. wet	$\operatorname{Control} \operatorname{O}_2$	$\mathbf{E}_{\mathbf{x}}$ $\mathbf{p}_{\mathbf{c}}$	rimental ${ m Total~CO}_2$	Fermen- tation $CO_2^a$	- Percentage inhibition of resp.	R.Q
0	60	300		102.5	90.7			0.88
0	60	400		101	82.3			0.8
0	60	350		77.7	64.5			0.8
0	50	500		95.8	82.6	•••		0.8
0	50	350	• • • •	92.3	76.5			0.8
0	50	500		76.4	67.2			0.8
$1.0 \times 10^{-6}$	50	500	122	115	90.9		8.2	0.8
$1.0 \times 10^{-5}$	60	500	(122)b	124	91.0		0	0.7
$1.0 \times 10^{-5}$	50	350	96.0	94	80.4		2.1	0.8
$1.0 \times 10^{-4}$	50	350	(96.0)	58.5	49		39	0.8
$1.0 \times 10^{-4}$	60	350	75.0	44.5	37.5		40.7	0.8
$1.0 \times 10^{-4}$	60	350	(75.0)	35.7	34.0	3.7	52.4	0.9
$2 \times 10^{-4}$	60	350	78.0	16.4	41.0	27.9	79	2.5
$2  imes  imes 10^{-4}$	60	350	(78.0)	20.9	39.5	21.7	72	1.8
$3.0 \times 10^{-4}$	60	400	102	24.5	57.9	37.1	76	2.5
$3.0 \times 10^{-4}$	60	300	100	24.2	54.0	33.5	76	2.9
$8.0 \times 10^{-4}$	60	300	(100)	14.3	42.0	29.8	85	2.9
$1.0 \times 10^{-2}$	60	400	(102)	19.4	38.4	21.9	81	1.9

<sup>&</sup>lt;sup>a</sup> Calculated by subtracting experimental  $O_2 \times 0.85$  from total  $CO_2$ .

<sup>&</sup>lt;sup>b</sup> The parentheses indicate use of a single control vessel to calculate the percentages of inhibition for several poisoned samples in a single experiment.

to proceed at the normal anaerobic rate in air, unhindered by respiration. Upon transfer of the tissue to a poison-free medium, the aerobic fermentation returned practically to its normal respiration—limited value. Reference to table 7 shows that carrots

Table 7. Ethyl isocyanide and the Pasteur reaction.

Gas exchange in mm.3/gm. over 75 min.							
$ m O_2  m  ext{ Total CO}_2  m  ext{ F}$							
Control	155	158	1.02				
Experimental	151	159					
	136	148					
Average	143	153	1.07				

do not react in this way to ethyl isocyanide. If the Pasteur effect were poisoned by the isocyanide, the expected  $CO_2$  production in the presence of the poison would be the sum of the aerobic  $CO_2$  and the anaerobic  $CO_2$ . Since the anaerobic  $CO_2$  is 1.15 times the aerobic  $CO_2$ , the expected value in the poi-

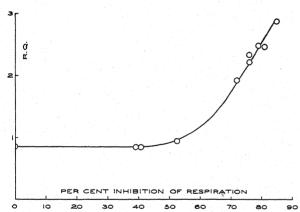


Fig. 1. The relation between R.Q. and respiratory inhibition by cyanide.

soned tissue is 2.15 the  $CO_2$  of the unpoisoned control, or  $2.15 \times 158 = 340$ . The experimentally determined value is 153.

Genevois (1927) has shown that in *Lathyrus* low concentrations of cyanide which do not inhibit respiration do induce aerobic fermentation, thus poi-

soning the Pasteur effect. Laser (1937a) has shown that in several animal tissues low oxygen pressures which do not lower the respiratory rate induce aerobic glycolysis. Further, Laser (1937b) has shown that in such tissues 80 per cent CO does not inhibit oxygen consumption but does poison the Pasteur effect, allowing aerobic glycolysis to occur. Inspection of table 6 shows that in cyanide concentrations from  $10^{-6}$  to  $1.0 \times 10^{-4}$  no aerobic fermentation occurs. Genevois' results with Lathyrus were not shown in carrots. The results listed in table 2 show that in 5 per cent oxygen with a respiratory inhibition of 27.5 per cent no fermentation occurs. Thus Laser's results with animal tissue could not be duplicated here. We have insufficient data on CO at various pressures to settle the problem of whether the Pasteur effect can be poisoned by CO in carrots. The fact that our results do not agree with those of Warburg, Laser, and Genevois, is not meant to question their results, but to point out differences in the Pasteur effect in different organisms.

#### SUMMARY

The results presented in this paper clearly demonstrate the existence of the Pasteur effect in carrot root tissue. Further, they show that with cyanide inhibition of respiration in excess of 45-50 per cent there is a direct effect of respiration on the inhibition of fermentation. Sodium azide and carbon monoxide also induce aerobic fermentation, and the carbon monoxide effect is reversed by light. These results are not consistent with the Lipmann theory of the oxidation of a fermentation enzyme unless the oxidation is catalyzed by cytochrome oxidase. However, they are consistent with, but give no proof for, the Meyerhof oxidative resynthesis theory. Direct proof of this theory for a higher plant may be obtained only by simultaneous analysis for carbohydrate, alcohol, CO2, and O2. Such information is not available for any single plant. The Pasteur effect is not poisoned by ethyl isocyanide, low oxygen pressures, nor by cyanide.

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## GROWTH SUBSTANCES IN AGAR 1

## William J. Robbins

ONE of the commonest substances employed to prepare a solid medium for the cultivation of microorganisms is agar-agar. It is made from marine algae, chiefly *Gelidium corneum* (Smith, 1905) and was first used by Frau Fannie Hesse in 1881 (Hitchens and Leikind, 1939); the first published notice of its use was by Koch (1882).

Added at concentrations of 0.5 per cent or more to solutions containing suitable foods and nutrients, it furnishes a solid or semi-solid substratum upon which yeasts, bacteria, and filamentous fungi may be grown. It is usually regarded as an inert agent, though some organisms have been reported which digest it (Goresline, 1933). Since it is considered primarily as a convenient means of solidifying a liquid medium, the amount employed depends chiefly upon the degree of solidity desired and upon convenience in filtration. Commonly added in amounts sufficient to make a 1.0 or 2.0 per cent solution, some prefer lower concentrations, and others have found superior results from media containing as much as 3.0 or 4.0 per cent.

Since agar is prepared from the algae by extraction with hot water, it contains a variety of inorganic salts and organic materials. Smith (1905) gives the following percentage analysis for a sample of kanten or crude agar: water, 22.29; protein, 6.85; fiber, 6.73; carbohydrates, 60.32 and ash 3.81. The agar used in preparing culture media is purified to various degrees, some of the ash and other materials being removed in the process, and preparations of different degrees of purity are available on the market. According to a letter from the Digestive Ferments Company, Difco agar contains on the average 0.29 per cent nitrogen and 3.0 per cent ash. According to Gortner (1929), agar is a sulfuric acid ester where the ester group is a complex polysaccharide and the gelatinization is that of a salt of an agar sulfuric acid.

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Observations that the growth of organisms on an agar medium is superior to that in a liquid medium have been reported from time to time. Robbins and White (1936) found agar or a water extract of agar to exert a favorable influence on the growth of excised corn roots. Lignières (1919) found 0.25 per cent agar favorable for the cultivation of anaerobes. Hitchens (1921) reported a beneficial effect of dilute agar (down to 0.05 per cent) upon the growth of aerobic and anaerobic bacteria. Lignières and Hitchens accounted for the beneficial action of the agar by its effect upon the oxygen tension in the medium. Itano and Tuiji (1934) found that agar of lower grade supported the better growth of Azotobacter, B. subtilis, and Saccharomyces cereviseae. They believed that this was caused by a larger quantity of some stimulating substance—for example, iodine-in the cruder agar. Umbreit (1939) observed growth of species of Proactinomyces to be better on agar media than on corresponding liquid media. He believed the benefit to be a surface effect acting indirectly through perhaps the oxidation-reduction potential. The action of agar might be ascribed to various causes including the inorganic substances it contains, especially the trace elements, its adsorptive power because of its colloidal character, a favorable effect upon the hydrogen-ion concentration, a physical effect upon growth, its effect upon the oxygen supply, its nitrogen content, its action in lowering the surface tension or the presence of growth substances. The present paper presents evidence that agar exerts a favorable effect upon Phycomyces Blakesleeanus and that this effect is probably because of the presence in commercial agar of growth substances.

MATERIALS AND METHODS.—The strain of *Phycomyces Blakesleeanus* used was originally secured from Dr. A. F. Blakeslee. Petri dishes 10 cm. in diameter and 1.5 cm. deep were used as culture vessels. Sterile conditions were maintained throughout; the liquid or agar media were sterilized in an autoclave at 15 lbs. pressure for 20 minutes. The *Phyco-*

Table 1. Spore germination of Phycomyces on Difco agar and on the same agar purified by extraction with aqueous pyridine. Counted after 20 hours. All media contained same amounts of dextrose, asparagine, mineral salts, and thiamin.

Medium	No. spores counted	No. spores germ.	Percentage germ.	Approximate length of hyphae, mm.
1.0% Agar	1,103	45	4.0	0.60
1.0% Purified agar	1,165	14	1.2	0.12
0.5% Agar		20	1.8	0.35
0.5% Purified agar		7	0.5	0.12

myces cultures were incubated at 25°C. in weak diffuse light. Distilled water was used in preparing the media, and the chemicals were of chemically pure grade. The thiamin was Merck's synthetic.

Two basic culture solutions were used, each of which was supplemented with 0.01 ppm. boron, 0.02 ppm. molybdenum, 0.20 ppm. iron, 0.15 ppm. zinc, 0.04 ppm. copper and 0.02 ppm. manganese. Solution 1 contained per liter 50 g. dextrose, 0.5 g. asparagine, 0.5 MgSO<sub>4</sub> · 7 H<sub>2</sub>O, 1.5 g. KH<sub>2</sub>PO<sub>4</sub>, and 0.25 mg. thiamin. Solution 2 contained per liter 100 g. dextrose, 1.0 g. asparagine, 0.5 g. MgSO<sub>4</sub> · 7 H<sub>2</sub>O, 1.5 g. KH<sub>2</sub>PO<sub>4</sub>, and 0.25 mg. thiamin.

EXPERIMENTAL.—Agar beneficially affects the development of *Phycomyces*. From macroscopic examination it appeared that evident mycelium developed in a liquid medium of mineral salts, asparagine, dextrose, and thiamin one or two days later than on the same medium to which 1.0 or 2.0 per cent agar was added. The difference in growth seemed less pronounced as the cultures became older. It might reasonably be suggested that the greater early development on the agar medium was caused by the better oxygen supply available to the organism on the surface of the solid medium as compared to that in a

liquid medium where the spores and early mycelial growth were submersed.

However, such an explanation is inadequate because the beneficial effect of the agar increased with its concentration and the organism grew in these instances on the surface with similar access to oxygen. Spore germination, growth, especially early growth, and the formation of gametes and zygotes were all beneficially affected by increasing the concentration of agar.

For example, to solution 1 sufficient Difco agar was added to make a 0.5 per cent and a 1.0 per cent solution. Approximately 25 ml. of these media were poured into Petri dishes, and by means of a platinum loop drops of a spore suspension of *Phycomyces* in sterile distilled water were placed on the surface of the solidified agar. Spore counts made after 18 hours' incubation at 25°C. showed 4.0 per cent germination on the 1.0 per cent agar and 1.8 per cent on the 0.5 per cent agar. The hyphae arising from the spores on the 1.0 per cent agar were on the average about twice as long as those on the 0.5 per cent agar (table 1).

The effect of agar concentration on growth may be illustrated by the following experiment. In this

Table 2. Colony diameters and dry weights of Phycomyces Blakesleeanus grown in Petri dishes on Difco agar and on same agar purified by extraction with aqueous pyridine. All cultures contained same amounts of dextrose, mineral salts, and thiamin.

		Co	lony dian	neters in mn	Dry wt. mg. per culture			
-	44 h	nours	62 1	hours	110	110 hours 110 h		hours
Concn. of agar	Difco agar	Purified	Difco agar	Purified	Difco agar	Purified	Difco agar	Purified
0.5	2.5	1.2	4.8	3.6	8.2	7.2	41.0	16.7
1.0	2.8	1.7	5.4	3.6	8.8	7.7	32.3	18.7
2.0	3.3	1.5	5.9	3.5	8.9	7.3	34.7	14.0
3.0	3.5	2.0	5.7	3.3	8.9	7.1	39.3	19.3
4.0	3.3	1.9	6.0	3.6	9.0	- 6.7	42.7	18.0
			0.0	5% Aspara	gine			
0.5	1.9	2.0	4.1	3.3	8.6	7.1	102.0	66.7
1.0	2.4	2.0	5.0	3.0	9.0	6.5	157.0	53.3
2.0	2.6	1.2	5.4	3.4	9.0	7.3	175.0	75.0
3.0	2.8	statienia.	4.8	4. ja	9.0		190.0	
4.0		1.6		3.2	••	6.0	••••	66.0
			0.	4% Aspara	gine			

instance solution 1 containing 0.05 per cent asparagine and the same solution containing 0.4 per cent asparagine were used. With each of these solutions media containing 0.5 per cent, 1.0 per cent, 2.0 per cent, 3.0 per cent, and 4.0 per cent agar were prepared, poured in Petri dishes, and inoculated in the center of each dish with a drop of a spore suspension of the plus strain of *Phycomyces*. Triplicate plates were used for each agar concentration and each asparagine concentration. In addition to general observations colony diameters were measured at intervals, and after 110 hours the agar was melted,

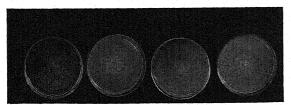


Fig. 1. Effect of concentration of agar on growth of *Phycomyces*. From left to right 1.0 per cent agar, 2.0 per cent, 3.0 per cent, 4.0 per cent. All contained per liter 50 g. dextrose, 0.5 g. asparagine, 0.5 g. MgSO<sub>4</sub> · 7 H<sub>2</sub>O<sub>7</sub>, 1.5 g. KH<sub>2</sub>PO<sub>4</sub>, 0.25 mg. thiamin and mineral supplements. Age 63 hours.

mycelium washed in hot water, and dry weights were determined by drying at 100°C. (table 2).

Growth on the 0.5 per cent agar after 44 hours was rather sparse; it increased as the concentration of agar increased, the heaviest growth appearing on the highest concentration of agar. The difference was more marked between that on the 1.0 per cent and on the 2.0 per cent agar than between that on other successive steps in the concentrations used (fig. 1). The growth on the media containing the larger amount of asparagine was somewhat less extensive though perhaps heavier. Colony diameters (table 2) increased with the concentration of agar, though the differences in growth were greater than indicated by the colony diameters, especially between the growth at 0.5 per cent and 1.0 per cent agar and that at higher concentrations, because colony diameters do not measure density. The measurements of colony diameters at the end of 62 and 110 hours showed similar effects of increasing concentration of agar. As the colonies aged, the heaviness of the mat increased on the more dilute agars so that the appearance was more nearly alike on all concentrations than in the first day or two of development. The dry weights at the end of 110 hours (table 2) showed the effect of the agar concentration more markedly with the larger amount of asparagine.

The production of gametes and zygotes by *Phycomyces* is influenced by a number of factors, including the concentration of agar. On solution 1 containing 1.0 per cent agar gametes were rarely produced if the plates were inoculated with a drop of a spore suspension of the plus strain at one edge and the minus strain at the other so that the distance between the two drops was about 8.5 cm. If the agar

concentration was increased to 2.0 per cent, a yellowish line of gametes formed, and a few zygotes with appendages developed. With 3.0 per cent agar the thickness of the line of gametes and the number of zygotes with appendages was still greater. For example in 4 Petri dishes of solution 1 with 1.0 per cent agar inoculated with plus and minus strains as described above no gametes were produced. With the same solution containing 3.0 per cent agar a yellowish line of gametes was formed, and an average per plate of 62 zygotes with appendages was counted (fig. 2).

Of the various explanations which might be offered for the beneficial effects of agar described above, oxygen supply for the reasons discussed earlier does not appear a probable one. Although Difco agar contains some nitrogen growth of Phycomyces on a Difco agar medium without added nitrogen but containing sugar, mineral salts, and thiamin was extremely scant indicating that the nitrogenous compounds in the agar are not in an available form or in sufficient amount to be significant. Furthermore, the beneficial action of increased concentration of agar was evident when the asparagine was raised to 0.4 per cent. It appears unlikely that mineral elements are concerned, since a group of micro-elements was added to the solution cultures and to the various concentrations of agar. Changes in hydrogen-ion concentration also do not appear significant, since the KH<sub>2</sub>PO<sub>4</sub> added would be expected to give much the same reaction to the media in all the plates. The agar might adsorb some ions and better balance the

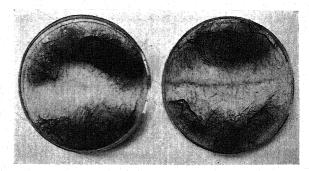


Fig. 2. Effect of concentration of agar on formation of gametes and zygotes by *Phycomyces*. Left. 1.0 per cent agar; right, 3.0 per cent agar. Both inoculated at opposite edges with drop of spore suspension. Both contained per liter 100 g. dextrose, 1.0 g. asparagine, 0.5 g. MgSO<sub>4</sub>· 7 H<sub>2</sub>O, 1.5 g. KH<sub>2</sub>PO<sub>4</sub>, 0.25 mg. thiamin and mineral supplements. Age 11 days.

solution or it might affect development through its physical characters which would change somewhat with concentration. It appears, however, that these possibilities also may be eliminated, because it was found that beneficial materials could be removed in part by extraction with methyl alcohol or with aqueous pyridine.

In an attempt to extract the beneficial material, 5 g. of Difco agar were dissolved by heating in 100 ml. of distilled water. The hot liquid was poured

with stirring into 200 ml. of redistilled methyl alcohol. The precipitate was centrifuged off, redissolved in hot water and reprecipitated with alcohol. The precipitate was again removed by centrifuging, washed with three 30 ml. portions of 60 per cent methyl alcohol, and dried. The methyl alcohol extracts were combined and evaporated by boiling to small volume. About one-half of the original 5 g. of agar was recovered in the precipitate, the balance remaining in the methyl alcohol extract. Media were prepared consisting of solution 2 containing 1.0 per cent Difco agar, 1.0 per cent agar purified by extraction with methyl alcohol, or the methyl alcohol extract sufficient to make 1.0 per cent agar and poured into Petri dishes. Triplicate plates of each medium were inoculated with three drops of a spore suspension of the plus strain of Phycomyces arranged in a line across the plate and three drops of a spore suspension of the minus strain arranged in a parallel line 2.5 or 3 cm. from the first. The mycelial growth was most rapid on the plates containing 1.0 per cent Difco agar and considerably less rapid on those plates containing the purified agar. The growth on

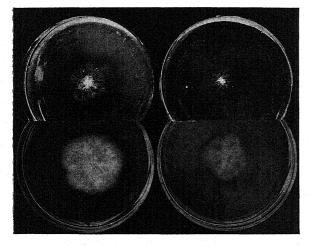


Fig. 3. Effect of purification of agar on growth of *Phycomyces*. Left, 0.5 per cent Difco agar; right, 0.5 per cent Difco agar purified with pyridine. Above at end of 48 hours; below, 63 hours. All contained per liter 50 g. dextrose, 0.5 g. asparagine, 0.5 g. MgSO<sub>4</sub> · 7 H<sub>2</sub>O, 1.5 g. KH<sub>2</sub>PO<sub>4</sub>, 0.25 mg. thiamin and mineral supplements.

the methyl alcohol extract was better than that on the purified agar and not as good as that on the unpurified. Under the conditions of inoculation described a medium heavy line of yellowish gametes developed on the unpurified agar plates; on the purified agar the line was thin (fig. 6); on the agar extract it was intermediate between the two given above.

It appeared from this experiment that materials beneficial to the development of *Phycomyces* were present in Difco agar and that they could be extracted in part with dilute methyl alcohol. However, the extraction was only partially successful and considerable agar was lost in the process. An attempt

was therefore made to extract the agar with 5.0 per cent aqueous pyridine.

Fifty grams of powdered Bacto agar were extracted at room temperature over a period of 48 hours with 500, 300, and 500 ml. of 5.0 per cent aqueous pyridine. The extracts were combined, the pyridine distilled off, and the solution evaporated to 100 ml. The extract contained 2.5 per cent solid material. The agar was washed twice with 95 per cent ethyl alcohol and air dried. More than 45 g. of the original 50 g. were recovered.

Solution 1 and the same solution with 0.4 per cent asparagine were prepared with 0.5 per cent, 1.0 per cent, 2.0 per cent, 3.0 per cent and 4.0 per cent of the purified agar poured into Petri dishes and inoculated in the center of each plate with a drop of a spore suspension of the plus *Phycomyces*. Triplicate plates were used with each agar concentration.

Colony diameters were measured and dry weights determined. The growth on the purified agar was distinctly poorer than on the unpurified agar (table 2, fig. 3). For example, at the end of 44 hours the colony diameter on 0.5 per cent Difco agar was 2.5 mm.; on the purified agar 1.2 mm.; at the end of 110 hours the dry weight on the 0.5 per cent Difco agar was 41.0 mg., on the purified agar 16.7 mg. The differences between the growth on the purified and unpurified agar were evident on the media containing 0.05 per cent asparagine and on those containing 0.4 per cent asparagine. Increasing the concentration of the purified agar had less effect than increasing the concentration of the unpurified agar.

It appeared from these experiments that agar extracted with aqueous pyridine was less favorable for the growth of *Phycomyces* than unextracted agar. That this was because of the removal by the extraction of some beneficial factor or factors and not an effect caused by a residuum of pyridine or the development of injurious products by the treatment was demonstrated by the effect of the addition of the extract to the purified agar. In this instance *Phycomyces* was grown in Petri dishes as before on solution 1, plus 1.0 per cent purified agar, and on the same medium plus the pyridine extract equivalent to 1.0 per cent, 2.0 per cent and 4.0 per cent agar. The growth on the media to which the extract was

Table 3. Colony diameters and dry weights of Phycomyces Blakesleeanus grown on Difco agar purified by extraction with aqueous pyridine and same agar plus different amounts of extract. All cultures contained same amount of dextrose, asparagine, mineral salts, and thiamin.

	Colony	r in mm.	Dry wt. mg.		
1% Purified agar plus	44 hrs.	62 hrs.	110 hrs.	110 hrs.	
Nothing Extract equivalent to		3.6	7.7	18.7	
1% agar Extract equivalent to	2.1	4.8	8.9	27.3	
2% agar Extract equivalent to	2.9	5.5	9.0	39.0	
4% agar		6.3	9.0	40.0	

added was considerably better than that on the purified agar alone (table 3, fig. 4) and almost as good as that on the original Difco agar (compare with table 2). Gamete production also was nearly as good on the purified agar plus the extract as on the un-

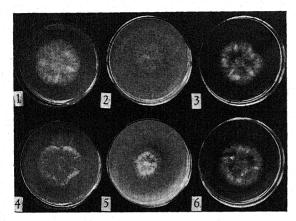


Fig. 4. Purification of agar and growth of *Phycomyces*. 1, 2.0 per cent Difco agar; 2, 2.0 per cent Difco agar purified by extraction with aqueous pyridine; 3, 1.0 per cent purified agar plus extract equivalent to 2.0 per cent agar; 4, 4.0 per cent Difco agar; 5, 4.0 per cent Difco agar purified by extraction with aqueous pyridine; 6, 1.0 per cent purified agar plus extract equivalent to 4 per cent agar. All contained per liter 50 g. dextrose, 0.5 g. asparagine, 0.5 g. MgSO<sub>4</sub> · 7 H<sub>2</sub>O<sub>7</sub>, 1.5 g. KH<sub>2</sub>PO<sub>4</sub>, 0.25 mg. thiamin and mineral supplements. Age 63 hours.

purified Difco agar (fig. 5). From these experiments it was concluded that agar contains some substance or substances which favor spore germination, growth, and the production of gametes and zygotes by *Phycomyces*. These substances can be extracted from the agar by aqueous pyridine.

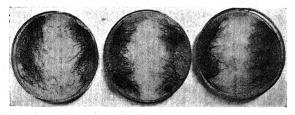


Fig. 5. Purification of agar and gametic reproduction by *Phycomyces*. Left, 1.0 per cent Difco agar; center, 1.0 per cent Difco agar purified by extraction with aqueous pyridine; right, purified agar plus extract equivalent to 1.0 per cent agar. All contained per liter 50 g. dextrose, 0.5 g. asparagine, 0.5 g. MgSO<sub>4</sub> · 7 H<sub>2</sub>O, 1.5 g. KH<sub>2</sub>PO<sub>4</sub>, 0.25 mg. thiamin and mineral supplements. Plates inoculated in 6 places in two lines 2.5 cm. apart.

Substances having similar effects are present in brown sugar, potato, corn meal, and oatmeal. A detailed report of the effects of these substances on *Phycomyces* will not be made here. Their effects are marked. For example, in one experiment the percentage of germination of *Phycomyces* on solution 1 containing 1.0 per cent Difco agar was 3.6 per cent

at the end of 22 hours and 5.5 per cent at the end of 46 hours. On the same medium to which was added a concentrated extract of white potato equivalent to 2.75 g. of potato per plate the germination was 22.0 per cent and 30.5 per cent at the time intervals indicated. Furthermore, the addition of potato extract to the agar purified with methyl alcohol or aqueous pyridine increased the production of gametes and zygotes (fig. 6).

What are the beneficial substances in the agar? It appears probable that they are growth substances rather than minerals or sources of nitrogen, since both the latter seem to be eliminated for the reasons given above. That agar contains thiamin or its intermediates and biotin was demonstrated by the growth of *Phycomyces* and of *Ashbya* (Robbins and Schmidt, 1939). Development of the *Ashbya* in a

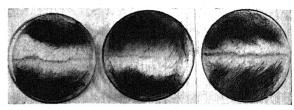


Fig. 6. Effect of purification of agar and potato extract on gametic reproduction by *Phycomyces*. Left, 1.0 per cent Difco agar; center, 1.0 per cent Difco agar purified by methyl alcohol; right, 1.0 per cent purified agar plus potato extract equivalent to 2.75 g. potato per plate. All contained per liter 50 g. dextrose, 0.5 g. asparagine, 0.5 g. MgSO<sub>4</sub> · 7 H<sub>2</sub>O<sub>4</sub>, 1.5 g. KH<sub>2</sub>PO<sub>4</sub>, 0.25 mg. thiamin and mineral supplements. Inoculated in 6 places in two lines 2.5 cm. apart.

medium supplemented with an agar extract made with aqueous pyridine indicated the presence of 0.041 gamma of biotin per g. of agar, and the growth of *Phycomyces* in a medium lacking thiamin but supplemented with the agar extract indicated the presence of traces of thiamin or its intermediates.

If a growth substance is concerned in the favorable effects of agar described above, it is not thiamin or its intermediates, since an excess of this growth substance was added to the media. It is not vitamin B<sub>6</sub>, because the addition of a pure synthetic preparation of this compound to a medium containing thiamin had no marked beneficial effect upon either growth or the gamete formation of Phycomyces, which is contrary to the report by Sinclair (1938) that vitamin  $B_6$  has a favorable effect in the presence of thiamin. It would not seem to be biotin, since Kögl and Fries (1937) found biotin ineffective on the growth of Phycomyces in the presence of thiamin. Other growth substances which might be suspected could not be tested, because they were not available in pure form.

Discussion.—While in our opinion these experiments demonstrate the presence in agar of growth substances beneficial to the development of *Phycomyces*, final and conclusive proof of this must wait the identification of the substances concerned and the demonstration of their effects in pure form. This

must be borne in mind in the following discussion which is based on the assumption that we are dealing

with growth substances.

On that basis it follows that Phycomyces requires for maximum development a growth substance other than thiamin. This unknown exists in agar, potatoes, corn meal, oatmeal, brown sugar, and doubtless many other materials of natural origin. While Phycomuces is unable to synthesize thiamin and must be supplied with that growth substance or its intermediates if it is to grow at all, we believe it makes some of the unknown material, though insufficient for maximum development. The situation is somewhat analogous to that which exists for the strain of excised tomato roots with which we have worked (Robbins and Schmidt, 1939a, 1939b). The tomato root also makes no thiamin and will not grow without an external supply of thiamin (or thiazole). In the presence of thiamin, growth of the tomato root is limited by its ability to synthesize vitamin B6, and until the amount of this substance made by the root is supplemented by an outside supply growth is slow, even in the presence of thiamin. With Phycomyces the second growth substance is not vitamin B<sub>6</sub> but another as yet unknown. It is entirely possible, of course, that deficiencies of more than one unknown growth substance are involved in the development of this fungus.

Even though the experiments described here have been concerned primarily with *Phycomyces*, it is probable that other organisms also will be found to respond favorably to the growth substances in agar. The beneficial effects of an agar extract on *Ashbya* and on excised corn roots have already been cited. At the same time organisms will doubtless be found which will not evidence any benefits. This would be in accordance with the concept we have formed of the relation of growth substances to organisms. Many are essential for the development of any organism. Some organisms form all in amounts adequate for growth; others lack the ability to make one or more than one in sufficient amounts, and the de-

ficiencies vary with the organism.

It is of some interest to note that for *Phycomyces* a critical point seemed to be between 1.0 per cent and 2.0 per cent Difco agar. A concentration of 1.0 per cent and below was much less effective than 2.0

per cent and above. This would suggest that for the media used the amount of growth substance in the former concentration is inadequate and in the latter nearly adequate. This critical concentration would of course vary with the purity of the agar. Dr. B. O. Dodge has advised us that he has long had the impression that Difco corn meal agar made with 2.0 per cent agar is more favorable for the perthicial development *Neurospora* than the same medium made with 1.0 per cent agar.

In any event it seems clear that agar as a medium for investigations of the growth of organisms and particularly for investigations on growth substances cannot be regarded as an inert material; it is necessary to take into consideration the possibility that it may influence results because of the growth substances it contains. At the same time it appears also that thiamin is not the only growth substance which *Phycomyces* requires from without, and experiments in which this organism is employed must take that

fact into consideration.

#### SUMMARY

A beneficial effect of Difco agar upon the spore germination, the growth and the formation of gametes and zygotes by Phycomyces Blakesleeanus was observed. The beneficial material could be extracted in part from the agar with dilute methyl alcohol and more completely with aqueous pyridine. Since the media used contained minerals (including micro-essential elements), sugar, asparagine, and thiamin, it is believed the beneficial action of the agar was caused by unknown growth substances. Agar was found to contain appreciable quantities of biotin and traces of thiamin or its intermediates. Potato, brown sugar, oatmeal, and corn meal favorably affected the development of Phycomyces in the presence of thiamin and probably contain the same unknown growth substances believed to be in agar. The possibility of the occurrence of growth substances in agar is a factor which must be considered in using that material in experiments with living organisms.

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Physiol. Chem. 249: 93-110.

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# STIMULATION OF GROWTH IN ASPERGILLUS NIGER UNDER EXPOSURE TO LOW VELOCITY CATHODE RAYS 1

C. E. Buchwald and R. M. Whelden

STIMULATION OF the growth of unicellular organisms under exposure to low dosages of various ionizing radiations has been rather frequently reported in the literature.2 In particular, stimulation of growth of yeasts and molds in wide variety has been reported when these organisms have been exposed to very low dosages of X-rays, radium emanations, and ultraviolet radiation. This effect, on the other hand, has been denied by many workers—a fact which seems to warrant the publication of this paper.

For several years we have been engaged in a study of the various effects which may be obtained when spores of the Ascomycete fungus Aspergillus niger are exposed in high vacuum low voltage cathode ray beams. This work has been partly reported elsewhere (Buchwald and Haskins, 1938; Whelden, 1938; Haskins, 1938; Cooper, et al., 1939).

Early in the course of this work it was observed that after irradiation at certain voltages and currents there was a marked acceleration in the rate and in the extent of swelling of the spores. Because of the uniqueness of the ionizing radiation, the high degree of uniformity of the biological material, the very large amount of data taken, and the number and character of statistical tests which have been applied, we feel that we have been able to demonstrate conclusively the occurrence of stimulation.

Source and character of radiation and radia-TION PROCEDURE.—The low voltage cathode ray tube which was used in this work has been rather fully described elsewhere (Buchwald and Haskins, 1938; Haskins, 1938; Cooper, et al., 1939). It consists essentially of an electron gun for the acceleration of electrons from a hot filament and a chamber in which the spores are placed for the irradiation process. Auxiliary apparatus evacuates the gun and chamber, supplies the high voltage for accelerating the electrons, and measures the number of electrons striking the organisms. The combined assembly, as it appears during a run, is shown in figure 1.

Within the raying box is a rotatable sample holder (fig. 2) upon the periphery of which can be clipped twenty-three small brass slides, chromium plated and highly polished. Spores from a culture of the fungus at least six weeks old are carefully spread

<sup>1</sup> Received for publication August 3, 1939.

<sup>2</sup> Chavarria and Clark, 1924; Hutchinson and Newton, 1930; Luyet, 1932; Nadson, 1925; Nadson and Philippov, 1928; Smith, 1935; Zeller, 1926.

upon these slides with a camel's hair brush, so that each spore is separated from its neighbors, on the average, by a distance five to ten times its own diameter. The slides are clipped into position on the rotating cylinder, the raying chamber is sealed and evacuated to a pressure of the order of 10<sup>-5</sup> mm. of mercury, and the spores are scanned by the beam from the electron gun as they travel across the slit system connecting gun and raying box. A Faraday cage, mounted within the cylinder, permits an accurate measure of the total number of electrons which strike each slide, and this number can be varied within wide limits. When all the slides have been treated, air is admitted to the raying box and the electron gun, the slides containing the spores are removed, and the spores are "printed" onto plates of potato-maltose agar by gently pressing the slide face to the agar surface and removing it immediately. Great care was constantly taken to prevent any disturbance of the spores while on the surface of the slides, particularly when air was admitted to the apparatus. Care was also taken that all spores used should be of nearly uniform age.

CULTURING AND COUNTING PROCEDURE.—Cultures were started from all samples of a given run simultaneously. The agar plates were incubated for from 6 to 7.5 hours, out of direct sunlight, at 25°C.-28°C. Only a small central portion of the slide was scanned by the electron beam. The irradiated strip took the form of a band with sharp edges, extending at right angles to the long axis of the slide. The spores on the end of each slide therefore constituted a control for that slide. In addition, an entire control slide was run for each twenty-three rayed ones. It was mounted inside the drum where it was entirely shielded from the electron beam.

When the spores had been transferred to the agar surface of the culture plates, small groups were marked in each set so that they could be readily distinguished at any time. These marked groups usually included about thirty spores so distributed that their growing germ tubes would not become entangled too quickly. Camera lucida drawings were then made of all the spores of these groups of spores at definite intervals of time until growth of the tangled hyphae made it impossible longer to follow the development of each spore. For any single group of spores this was usually not more than twelve to fourteen hours. In a few cases a selected group of spores became "lost" or unrecognizable during the period of observation. In such cases a group from the immediate vicinity was chosen as a substitute.

From the series of drawings so made, measurements were taken directly of the dimensions of each spore. Measurements were made to the nearest halfmicron. The series of measurements for each set of spores was tabulated separately and afforded a good comparison of the relative rates of change of the spore dimensions for the various sets throughout the observation period. Spores which were "inactive" (i.e., in which neither germination nor swelling occurred during the experiment) were not counted. The value taken for the diameter of each spore, and so listed, was in fact the average of two diameters, taken at right angles.

RESULTS.—Data of diameters from a typical group of spores are shown in table 1. Occasional figures in the table are missing, an omission caused by the fact that at the moment of that observation the spore was overlooked due to its position on the agar plate. From such tabulations as these, averages and probable errors were computed in the usual fashion, representing the average rate of change of cell diameter with time for each group of spores studied. including controls.

In figures 3 and 4 are shown plots of the average rate of change with time of two irradiated runs. compared with their control groups. Additional aver-

ages are shown below.

Discussion.—From a statistical study of the data and an examination of the curves, it becomes evident that (a) the swelling of the irradiated spores starts earlier in the culturing time of the spore for irradiated than control samples; (b) the average rate of swelling is higher for irradiated spores; (c) the size of the irradiated spores at any given time averages larger; (d) a larger percentage of irradiated spores have germ tubes.

It is evident that, if such far-reaching conclusions are to be drawn, copious data must be available, and must be very carefully analyzed (statistically). For this reason, a very large amount of data was taken. and no fewer than three tests were applied to it to determine its statistical significance: the binomial law (Fry, 1938); "Student's" t-test (Fisher, 1936), and Pearson's "Chi-squared" test (Frv. 1938; Fisher, 1936).

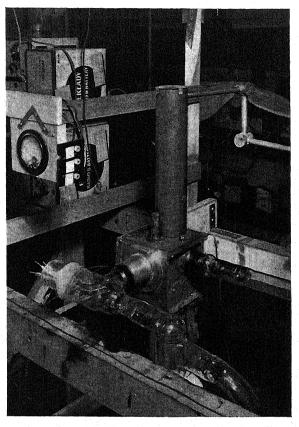


Fig. 1. General view of apparatus.

The binomial law is designed to give the probability that the observed grouping of the data can be accounted for entirely by chance. This test must be applied with discretion. If n represents the total number of times that the irradiated spores were measured and m is the number of measured values which are less than the control, then  $P_s = n!/$ 

Table 1. Hours after printing.

Spore no.	2.3	3.2	4.4	5.3	6.3	7.3	8.4	9.5	10.7	11.8	12.9	14.5
1	3.0	5.0	5.0	5.5	6.0	7.5	7.5	7.5	7.5	7.5	7.5	7.5
2	3.0	4.0	5.0	5.0	5.0	6.0	6.0	6.5	8.0	8.0	8.0	8.0
3	3.0	4.0	5.0	5.0		5.0	5.5	6.5	7.5	7.5	7.5	7.5
4	3.0	5.0	5.0	5.0	5.0	5.0	6.0	7.0	7.0	7.0	7.0	7.0
5	5.0	5.0	5.0	5.0	5.5	5.5	6.5	7.5	7.5	8.0	8.0	8.0
6	4.5	5.0	5.0	5.0	5.0	5.0	5.0	6.0	7.0	7.5	8.0	8.0
7	4.0										10.0	10.0
8			3.5	4.5	4.5			5.5			10.0	10.0
9		3.0	5.0	5.0	5.0	5.0	5.0	5.5	6.0	8.0	9.0	9.0
10		3.0	5.0	5.0	5.0	5.5	7.0	7.5	7.5	9.0	9.0	9.0
11	4.5	4.5	4.5	5.0	5.0	5.0	5.0	5.5	7.0	7.0	7.0	7.0
12	3.0	4.0	4.5	5.5	5.5	5.5	7.0	7.0	9.0	9.0	9.0	9.0
13	4.5	5.0	5.0	5.0	5.0	5.0	6.0	7.0	7.0	8.0	8.0	8.0
14	5.0	5.0	5.5	5.5	6.0	6.5	7.0	7.5	7.5	7.5	7.5	7.5

 $(n-m)!m!(\frac{1}{2})^n$ , where  $P_s$  represents the probability that the observed distribution of values of the data is a matter of pure chance alone. This test is useful in limited cases only.

"Student's" t-test is capable of yielding information concerning the significance of the observed statistical difference between the control and the irradiated material. It can tell the value of the probability that, had both groups of spores been treated similarly, the differences between them, arising purely from chance causes, would have been as great as

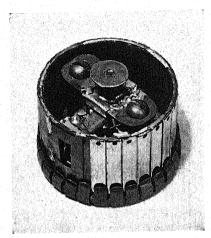


Fig. 2. Rotatable slide holder.

those observed. The smaller the value of this probability, of course, the more certain is it that the irradiated and the control spore populations differed because of their experimental treatment.

Pearson's "Chi-squared" test gives the same information as the t-test, but instead of being applied directly to the measurements of single spores or the average measurements of several of them, it is used to determine the theoretical frequency with which certain size-groups ought to appear. The two tests differ further in that the "Chi-squared" test can be applied to all the data representing a curve as well as to individual points on such a curve.

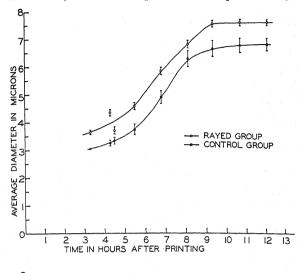
If the data which have been obtained in the work described can be shown to be significant by each of these three tests, there can be very little doubt that the effects of stimulation as they have been described are indeed real. It is therefore of great interest to apply each of these tests separately.

Table 2. Binomial law test.

Group		n	m	P's
A		7	0	.008
В	,	7	0	.008
C		9	0	.002
D		12	0	.00028
E		11	0	.00049
F		12	2	.016
G	<b>.</b>	5	0	.031
н		5	0	.031

In table 2 are shown the results of applying the binomial law to the sign of the differences for a given sample of data.

The application of "Student's" t-test, and, in some cases, of the "Chi-squared" to a representative



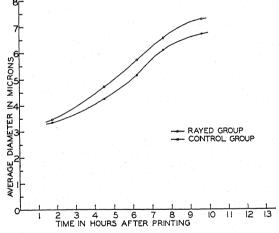


Fig. 3 (above). Fig. 4 (below).

sample of data are shown in tables 3, 4, 5, 6, 7, 8, 9, and 10. Here are listed the averages of eight spore groups at different times of culture, together with the averages of their controls, and the results of the t-test and in some cases of the "Chi-squared" test. The results of applying the "Chi-squared" test to the whole curve is also shown in some cases.

As a basis of comparison, three control groups have been divided arbitrarily into two parts and the t-test has been applied to these two groups, which were subjected to the same experimental conditions. The results are shown in table 11. Plots of the data shown in table 5 and table 10 are shown in figures 3 and 4.

It will be seen that, as shown by these three tests, the data are definitely significant, and the stimulation effects described are therefore confirmed.

Table 3. Data for Group A irradiated with  $44 \times 10^{-8}$  Coulombs per sq.cm. of 1.5 Kv. electrons.

		Time in hours after printing									
Data	4.6	5.7	7.0	8.2	9.4	10.8	12.3				
Average diameter of irradiated spores	{ 3.38  ±.06	4.50 ±.08	5.75 ±.11	6.77 ±.16	7.12 ±.15	7.44 ±.15	7.52 ±.14				
Average diameter of control spores	${3.36} \ \pm .13$	$3.85 \pm .19$	5.22 ±.24	6.34 ±.29	$6.69 \pm .29$	6.84 ±.26	6.89 ±.22				
"Student's" Test P	.92	.02	.13	.36	.35	.17	.11				
Chi-squared P	<.001	<.001	<.0001	.47	.20	.01	<.01				

No. of spores in rayed group 24; in control 10. P <.00003 for Chi-squared test on all data.

Table 4. Data for Group B irradiated with 264 × 10-8 Coulombs for sq.cm. of 1.5 Kv. electrons.

	Time in hours after printing									
Data	3.6	5.1	6.2	7.4	8.6	9.9	11.6			
Average diameter of irradiated spores	{ 3.25 ±.05	3.67 ±.07	4.51 ±.08	5.77 ±.10	6.80 ±.09	7.33 ±.07	7.41 ±.05			
Average diameter of control spores	${3.10} \\ \pm .04$	$\frac{3.56}{\pm .17}$	4.18 ±.19	5.74 ±.28	$6.52 \pm .30$	6.75 ±.28	$6.87 \pm .24$			
"Student's" Test P	.30	.65	.22	.92	.44	.06	.026			
Chi-squared P	<.01	.09	<.001	<.01	<.01	<.001	<.001			

No. of spores in rayed group 33; in control 10. P <.00003 for Chi-squared test on all data.

Table 5. Data for Group C irradiated with  $88 \times 10^{-8}$  Coulombs per sq.cm. of 1.5 Kv. electrons.

	Time in hours after printing									
Data	3.3	4.3	4.5	5.5	6.8	8.1	9.3	10.7	12.1	
Average diameter of irradiated spores	{ 3.67 {±.08	4.35 ±.11	3.72 ±.13	4.60 ±.12	5.86 ±.13	6.84 ±.14	7.58 ±.12	7.66 ±.12	7.69 ±.12	
Average diameter of control spores	$\begin{cases} 3.05 \\ \pm .02 \end{cases}$	$3.26 \pm .10$	3.34 ±.12	$3.76 \pm .19$	$\frac{4.92}{\pm .23}$	$6.30 \\ \pm .29$	6.68 ±.29	$6.82 \pm .26$	$6.89 \pm .23$	
"Student's" t test P	<.0001	<.0001	.22	.011	.013	.21	.025	.028	.026	
Chi-squared P	<.0001	<.0001	<.01	<.01	<.001	.25	.35	.30	.25	

No. of spores in rayed group 19; in control 10. P <.00003 for Chi-squared test on all data.

Table 6. Data for Group D irradiated with 130 × 10-8 Coulombs per sq.cm. of 3 Kv. electrons.

		Time in hours after printing										
Data	2.3	3.2	4.4	5,3	6.3	7.3	8.4	9.5	10.8	11.8	12.9	14.5
Average diameter of irradiated spores	${3.86} \pm .17$	4.37 ±.14	4.85 ±.09	5.08 ±.05	5.21 ±.08	5.54 ±.15	6.12 ±.16	6.65 ±.14	7.37 ±.13	7.84 ±.12	8.25 ±.17	8.25 ±.17
Average diameter of control spores	{ 3.80 ±.08	4.02 ±.09	4.36 ±.09	4.62 ±.10	5.00 ±.08	5.36 ±.08	5.80 ±.10	6.23 ±.11	$6.68 \\ \pm .10$	6.96 ±.07	7.11 ±.06	7.18 ±.06
"Student's" t test P	.99	.15	.015	.018	.24	.46	.25	.12	.0042	<.0001	<.0001	<.0001
Chi-squared P	<.01	<.01	.08	<.01	.75	.24	.50	.05	.30	<.01	<.01	<.01

No. of spores in rayed group 12; in control 20. P < .0001 for Chi-squared test on all data.

Table 7. Data for Group E irradiated with 220  $\times$  10-8 Coulombs per sq.cm. of 3 Kv. electrons.

				Tir	ne in hou	rs after	"printing	ıg"			
Data	2.40	3.27	4.50	5.37	6.47	7.45	8.53	9.62	10.88	11.98	13.37
Average diameter of irradiated spores	{ 4.32 }±.07	4.75 ±.04	4.95 ±.04	5.19 ±.05	5.65 ±.08	5.95 ±.10	6.26 ±.12	6.61 ±.14	7.05 ±.11	7.29 ±.07	7.41 ±.05
Average diameter of control spores	{ 3.82 }±.08	$^{4.04}_{\pm .09}$	4.39 ±.09	4.66 ±.10	5.04 ±.08	5.41 ±.08	5.85 ±.10	6.28 ±.11	6.72 ±.10	6.98 ±.07	7.14 ±.06
"Student's" t test P Chi-squared test for P			<.0001 <.01	<.001 <.01	<.0001 <.0001	.005 <.01	.085 .08	.22 <.01	.15 .01	.040	.02 <.01

No. of spores irradiated 22; in control 20. P <.00001 for "Chi-squared" test on all data.

Table 8. Data for Group F irradiated with 396  $\times$  10-8 Coulombs per sq.cm. of 3 Kv. electrons.

					Time in hours after printing							
Data -	2.58	3.50	4.75	5.62	6.72	7.68	8.73	9.87	11.10	12.32	13.67	15.00
Average diameter of irradiated spores	{ 4.05 } ±.10	4.64 ±.07	5.02 ±.05	5.09 ±.06	5.33 ±.12	5.50 ±.09	5.78 ±.12	6.61 ±.14	7.15 ±.14	7.47 ±.08	7.47 ±.08	7.50 ±.08
Average diameter of control spores	{ 3.86 } ±.08	4.10 ±.09	4.47 ±.09	4.75 ±.10	5.13 ±.08	5.50 ±.09	5.93 ±.10	6.38 ±.11	6.79 ±.09	7.04 ±.07	7.15 ±.06	7.18 ±.08
"Student's" t test P	0.33	<.001	<.001	.061	.36	1.00	(53)	.40	.15	.007	.034	.034
Chi-squared test P	<.001	<.0001	<.05	.03	.28	.55	.60	<.01	<.0001	<.0001	<.001	• • • • • • • • • • • • • • • • • • • •

No. of spores irradiated 18; of control 20. P < .00001 for "Chi-squared" test on all data.

Table 9. Data for Group G irradiated  $39 \times 10^{-8}$  Coulombs per sq.cm. of 3 Kv. electrons.

		Time in hours after printing						
Data	2.75	5.19	6.46	7.77	9.20			
Average diameter of irradiated spores	{ 4.18 }±.08	5.43 ±.06	6.37 ±.04	7.13 ±.04	7.58 ±.03			
Average diameter of control spores	{ 3.64 }±.02	5.34 ±.09	5.83 ±.07	$6.27 \pm .04$	$6.63 \pm .03$			
"Student's" t-test P. Chi-squared test P.	<.0001	.5 <.0001	<.0001	<.0001	<.0001			

No. of spores irradiated 287; of control 170.

Table 10. Data for Group H irradiated 39  $\times$  10-8 Coulombs per sq.cm. of 3 Kv. electrons.

	Time in hours after printing					
Data	1.77	4.41	5.94	7.46	9.45	
Average diameter of irradiated spores	{ 3.48 }±.014	4.74 ±.034	5.76 ±.043	6.59 ±.038	7.32 ±.029	
Average diameter of control spores	{ 3.36 }±.008	$4.27 \pm .025$	$5.16 \pm .036$	6.13 ±.035	$6.76 \pm .028$	
"Student's" t-test P	<.0001	<.0001	<.0001	<.0001	<.0001	

No. of spores irradiated 180; of controls 180.

Table 11. Control for Groups D, E, and F, arbitrarily divided into two parts.

Time in hours after printing "Student's" test P								
Deuta-		 	 0.01	0.01	0.00	0.01	1.00	0.01

Control for Group G arbitrarily divided into two parts.

Time in hours after printing 3.00 4.67 6.91 8.30 9.85 "Student's" t-test P...... 0.55 0.24 0.63 0.78 0.65

Control for Group H arbitrarily divided into two parts.

Time in hours after printing 2.15 4.83 6.41 7.81 9.96 "Student's" t-test P...... 0.20 0.99 0.01 0.41 0.53

The energies of cathode rays required to produce these stimulative effects were in general low, ranging from 1.5 to 3 kv. Such energies are no more than sufficient to propel the electrons through the cell wall and into the outer cytoplasm. The spherical, centrally-placed nucleus can hardly be involved. It follows that the stimulative effects which have been observed cannot be due merely to the fact that less hardy spores were completely killed, leaving only a sturdier population to grow, since cathode rays of these energies do not kill except in high density beams. The survival ratio of the irradiated material under these conditions may actually be as high as 120 per cent, as shown in table 12, giving data of the survival values for Aspergillus at 1.5 kv., 2 kv., and 3 kv.

cations may be primarily responsible for the change. It may be that a change in permeability of the cell wall is involved, resulting in a more ready absorption of water when the cell is "planted" on moist agar. More likely, there is an osmotic unbalance set up within the cell, caused by the large number of ions released within its substance, which may tend to draw water in through the cell membrane. It is also possible that some alteration of stored food material within the cell may have taken place which may make it more immediately usable in cell metabolism. Other explanations could doubtless be suggested.

#### SUMMARY

We have definitely been able to show that, when irradiated with cathode rays of certain energies and densities and subsequently cultured on potato-maltose agar, spores of the Ascomycete fungus Aspergillus niger are definitely stimulated in growth. Earlier signs of swelling, more rapid swelling, a larger average size at any given time, and a higher percentage of production of germ tubes have all been found in the irradiated samples as compared with controls.

A very large volume of data has been obtained in this connection, and three statistical tests of significance have been separately applied to it—the bino-

 ${\bf Table~12.~Survival~ratio~for~various~dos ages.}$ 

44 × 10-8 Coulombs/sq.cm. o 1.5 Kv. electrons	132 × 10-8 f Coulombs/sq.cm. of 1.5 Kv. electrons	175 × 10-8 Coulombs/sq.cm. of 2 Kv. electrons	88 × 10-8 Coulombs/sq.cm. of 3 Kv. electrons		
123.9	119.2	99.1	144.8		
103.2	96.2	98.6	151.4		
96.9	103.4	104.9	127.8		
107.0	105.3	139.2	152.8		
136.0	119.7	124.7	93.1		
122.6	124.1	120.4	92.9		
111.9	143.9	131.1	94.6		
115.3	136.8	92.4	86.9		
125.5	130.6	90.8	98.4		
109.9	117.5	97.1	104.1		
123.8	88.6	100.2	109.1		
121.8	108.2	110.6	98.6		

We can only speculate at this time as to the cause of this stimulative effect. Cathode rays, like ultraviolet and X-rays, dissipate their energy by ionization, but differ from those radiation agents in that their energy is lost over a much shorter range per electron than per quantum for X-rays or ultra-violet. There is little doubt that at the energies which we have used in this work by far the greatest portion of ionization was concentrated in the spore walls of the cells, extending to the membrane and part of the cytoplasm. The nucleus cannot be directly involved. It therefore seems probable that extra-nuclear modifi-

mial law, "Student's" t-test, and the "Chi-squared" test. The data have been shown to be significant by all these tests.

These stimulative effects are observed only at relatively low cathode ray energies, of the order of from 1.5-3 kv. No definite explanation of the effect can be attempted at present. Some suggestions have been made.

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# THE FORCES CONCERNED IN THE INTAKE OF WATER BY TRANSPIRING PLANTS <sup>1</sup>

## Paul J. Kramer

THE ABSORPTION of water is not an entirely independent process, but is one of several interrelated processes comprising the general field of plant water relations. The rates of transpiration, water conduction, and all the numerous other processes in which water plays a vital rôle are conditioned, at least in part, by the rate and amount of water absorbed through the roots. The rate of absorption, on the other hand, is affected by the rate of water loss, chiefly in transpiration, and the amount of water in the soil, its concentration, and its temperature. A complete discussion of the factors involved in water absorption requires some consideration of the structure of the roots, the laws governing the water relations of individual cells and those governing soil moisture relations. The present discussion will be confined to a consideration of the nature and origin of the forces operating to cause movement of water from the root hairs and epidermal cells across the intervening mass of living cells and into the xylem vessels.

Many discussions of the absorption of water by plants tend to stress the importance of the osmotic or secretory activity of the root cells but neglect the importance of the forces originating in the tops. It should be emphasized that the forces bringing about the intake of water originate not only in the roots but also in the shoots. In other words, some water is absorbed as a result of forces developed in the physiologically active root cells, but water is also absorbed because of forces developed in the tops by transpiration and transmitted to the roots.

During periods of low transpiration and abundant soil moisture, absorption by the roots often tends to

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exceed transpiration, resulting in the development of a positive hydrostatic pressure or "root pressure" in the xylem vessels. Since this type of absorption occurs only in the presence of physiologically active, living cells in the roots, it is frequently termed "active absorption" (Renner, 1915). The mechanism responsible for active absorption and the associated root pressure phenomena has not been satisfactorily explained, but it is usually regarded as some sort of osmotic phenomenon. Heyl (1933) after reviewing the extensive literature on the subject concluded that active absorption is an electro-osmotic process dependent on energy supplied by respiration of the root cells, a view also advanced by Lund (1931). Another explanation originated with the work of Atkins (1916) who suggested that root pressure is caused by diffusion of water from the very dilute soil solution across the cortical cells into the relatively more concentrated solution existing in the xylem vessels. Priestley (1922) believed that the endodermis, rather than the entire cortex, probably acts as the differentially permeable membrane. Kramer (1932) showed that water will diffuse through a thick, multicellular membrane of high osmotic pressure separating pure water from a sugar solution having an osmotic pressure much lower than that of the cells of the membrane. It was thus demonstrated that the cortex is capable of functioning as a membrane in the manner suggested by Atkins. None of these investigators was able to explain how a sufficiently high concentration of salts can be maintained in the xylem vessels to keep such a mechanism operating. Crafts and Broyer (1938) have suggested that the gradient of decreasing O2 and increasing CO2 existing from epidermis to stele in roots favors a higher level of activity in the cortical cells which are well supplied with oxygen than in the living cells of the stele. The cortical cells, therefore, are able to absorb salts at a comparatively high rate, but as the salts diffuse inward to the stele they are lost to the xvlem because the cells of the inner cortex and stele with a limited supply of oxygen and lower activity are unable to retain as high a salt concentration as the better aerated outer cortical cells. This would explain the maintenance of a sufficiently high concentration of solutes in the xylem to cause inward diffusion of water and perhaps root pressure. This scheme is complicated by the observations of Grossenbacher (1938, 1939) that cyclic variations in root pressure occur independently of any changes in temperature or light and are not related to variations in root respiration. Skoog, Broyer, and Grossenbacher (1938) have shown that the application of auxin to pea and sunflower seedlings stimulates exudation. This effect apparently is produced through the mechanism responsible for the diurnal periodicity in exudation, at least in sunflower. Possibly the diurnal variations actually occur in the metabolic process responsible for maintaining a supply of solutes in the xylem, and this in turn causes variations in exudation. These observations are difficult to reconcile with any theory based solely on a simple osmotic mechanism, and apparently we must still regard active absorption as incompletely explained.

The situation in the xylem of freely transpiring plants seems to be quite different from that in the xylem of slowly transpiring plants. During periods of moderate to rapid transpiration or low soil moisture, transpiration tends to exceed absorption, and positive pressure or exudation from cut stems cannot be demonstrated. On the contrary, water is usually absorbed through the cut surfaces of stumps of detopped plants, indicating that the water in the xylem is under reduced pressure or even tension. Removal of water from the xylem of the leaves by the mesophyll cells causes a reduced pressure or pull which is transmitted through the xylem to the roots, causing the movement of water from the surrounding living cells into the xylem. This movement occurs in part because decreasing the pressure on the water in the xylem, or placing it under tension, reduces its diffusion pressure. This increases the diffusion pressure deficit of the water in the xylem as compared to that of the water in the surrounding cells and in the soil. A gradient of decreasing vapor pressure and increasing diffusion pressure deficit is thus produced, along which water will diffuse from soil to xylem (Meyer and Anderson, 1939, p. 234 and 277). When an appreciable tension exists in the xylem, it seems possible that most of the water intake occurs as a mass flow along the gradient of decreasing pressure from soil to xylem. A gradient of less than one atmosphere, produced by a vacuum pump, greatly increases the intake of water, as has been shown by Renner (1929) and by Kramer (1932), and as is shown later in this paper. Under more extreme conditions the tension on the water in the xylem probably frequently exceeds the osmotic

pressure of the root cells, and the water in the cells also passes into a state of tension. At such times the "pull" of the transpiration stream presumably extends to the outer surfaces of the epidermal cells and is directly effective in bringing about water intake. During periods of rapid transpiration the absorption of water is determined principally by the rate of transpiration, and water intake is probably largely or entirely independent of the physiological or osmotic activity of the root cells. It has, therefore, been termed "passive absorption" by Renner (1915).

The importance of transpiration in bringing about the absorption of water was recognized even by some of the earlier physiologists. Hales (1727) carefully investigated the pressure with which water exuded from cut stems and measured the volume exuding but found that during the summer when transpiration was highest no exudation occurred. He believed that the loss of water in transpiration brought about the ascent of sap and stated, "And by the same principle it is, that we see in the preceding Experiments plants imbibe moisture so vigorously up their fine capillary vessels; which moisture, as it is carried off in perspiration (by the action of warmth), thereby gives the sap vessels liberty to be almost continually attracting of fresh supplies, which they could not do if they were fully saturate with moisture: . . . ." One might infer from these comments that Hales believed that transpiration was responsible for the conditions causing the intake of water. Sachs (1875, pp. 612-613) stated that root pressure could be of little importance in the ascent of sap, because when transpiration is rapid, water is absorbed by freshly cut stumps instead of exuding from them. Furthermore he found that the volume of water lost in transpiration from a leafy shoot always exceeded the volume of water exuding out of the stump from which the top had been removed. "From all this it still remains in doubt whether in such cases the contents of the cortical cells of the roots must not be left altogether out of consideration, since it is possible that the suction of the cell-walls merely, due to imbibition or surface action, reaches as far as the surfaces of the roots." It is clear that Sachs thought that the pull of transpiration, which he mistakenly believed to act largely through the cell walls, might bring about the absorption of water independently of any activity of the protoplasts of the root cells. Pfeffer (1900), who contributed much to our knowledge of osmotic phenomena, is even more explicit. Speaking of the pull of transpiration, he says (pp. 211-212), "By this backwardly transmitted suction the water in the roots is drawn into the vascular conducting channels, and at the same time the conditions necessary for fresh absorption from the soil outside are created by the agency of the cortex and by means of the root-hairs. No one-sided pumping action of the cortical cells is necessary for such absorption though if any does actually occur, it naturally aids and accelerates the latter. . . . That the consumption and removal of water by an organ should directly cause

absorption.

a fresh supply to be drawn in is a highly advan-

tageous arrangement. . . ."

Dixon (1910) stated that the tension developed in the water conducting system as a result of transpiration was transmitted to the roots and caused the intake of water by transpiring plants. He suggested that water might be regarded as moving through the plant along a gradient of decreasing vapor pressure from the soil to the parenchyma cells of the leaves. Renner (1912b) early distinguished between the absorbing forces developed in the roots and those developed in the top. Later Renner (1915) applied the

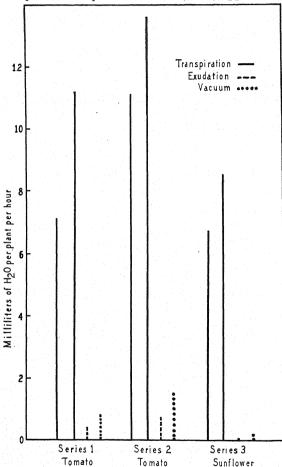


Fig. 1. Comparison of the rates of transpiration, exudation from detopped plants, and water movement through root systems attached to a vacuum pump. The plants were growing in soil kept near its field capacity. The lines for tomato represent averages of six plants, those for sunflower averages of eight plants.

term "active absorption" to water intake brought about by forces developed primarily in the roots and "passive absorption" to water intake brought about by the effects of transpiration. Livingston (1927) stated that the rôle of the osmotic forces and vital activities of the root cells in water intake had been greatly overestimated and emphasized the importance of the physical forces resulting from transpiration in bringing about water intake. Shull (1930)

believed that the tension in the water in the xylem of transpiring plants extends to the adjacent, living cells, causing movement of water from these cells into the xylem. In his opinion a gradient of water deficits is thus produced across the roots of transpiring plants, causing the intake of water.

In spite of these repeated assertions that the forces bringing about water intake by transpiring plants originate principally in the top, we still find writers emphasizing the osmotic properties of the root cells and the possible importance of root pressure, while more or less ignoring the probable importance of passive absorption. Possibly this is because a complex explanation of water intake by physiological absorption is more intriguing than a simpler explanation based largely on physical absorption. More probably it is because quantitative comparisons of the amount of water absorbed by the two mechanisms have seldom been made. In order to better evaluate the relative importance in the water economy of the plant of these two absorption mechanisms, a comparison was made of the amounts of water entering the plant by active and by passive

EXPERIMENTS AND RESULTS.—Experiments were performed on coleus (Coleus blumei Benth.), hibiscus (Hibiscus moscheutos L.), balsam (Impatiens sultani Hook.), sunflower (Helianthus annuus L.), and tomato (Lycopersicum esculentum Mill.). The plants were grown in four-inch pots in loam soil, which was wetted to approximately the field capacity at the beginning of each experiment. The pots were placed in metal containers which were covered with oil cloth to prevent evaporation and placed on a sunny bench in the greenhouse or out-of-doors. The transpiration rates were determined by weighing the plants in their containers. After determining the rates of transpiration for two or three hours, the tops were cut off about three cm. above the soil, and graduated pipettes were attached by means of rubber tubing and grafting wax. Enough water was placed in the pipettes to make the meniscus easily visible, and the volume of liquid absorbed or exuded was observed at quarter- or half-hour intervals. The plants were growing vigorously, the root systems were in excellent condition, and it is believed that the rates of exudation were as high as could be expected from plants of the size used. The results are shown graphically in figure 1. The rates of transpiration of the tomatoes are the average for six plants for two successive hour periods near the middle of bright days. The rates of exudation are also averages of six plants. The data for sunflowers represent the average of eight plants, and the exudation data were obtained later in the day from the same plants used for the transpiration experiment. It will be observed that the rate of exudation of the tomatoes amounted to only 3.6 to 5.4 per cent of the maximum rates of transpiration recorded, while that of sunflowers was less than 1 per cent of the rate of transpiration. The data for sunflowers represent the rate of exudation for an hour period beginning two hours after the conclusion of the transpiration measurements. At

Table 1. Comparison of transpiration and exudation in tomato. All rates are in ml. of  $H_2O$  per plant per half hour. The numbers under exudation indicate successive half-hour periods.

	Transp	iration	Exudation per half hour									
	1st hr.	2nd hr.	I		2	3	4	5	6			
1	8.0	8.9	a13		.02	.13	.20	.19	.18			
2	10.7	12.6	82		39	17	.04	.18	.20			
3	12.0	12.8	12		03	.05	.15	.19	.17			
4	10.8	9.3	-1.42		88	38	15	05	.03			
5	10.1	11.6	.17		.16	.25	.33	.32	.29			
6	8.2	9.8	23		08	04	.03	.07	.08			
Total	59.8	65.0	-2.55		1.20	16	.60	.90	.95			
		<b>1</b>										
		T	ops remov	red								

<sup>&</sup>lt;sup>a</sup> A minus sign indicates absorption of water through the stump instead of exudation from it.

this time two of the eight plants were still absorbing water instead of exuding it from the stumps. The rate of exudation of tomato was approximately doubled by attaching the tomato root systems to a vacuum pump and lowering the pressure until a gradient of 64 cm. of mercury existed from soil to the surfaces of the cut stems. The use of the vacuum pump increased the exudation of water from the sunflower about eightfold. As much water was obtained under vacuum from those sunflower root systems previously showing absorption through the cut surfaces as from those previously exuding freely.

A series of experiments was performed to study the rate of exudation from root systems following a period of rapid transpiration. A group of plants growing in pots of soil wetted to approximately the field capacity was allowed to transpire until noon on a sunny day. The tops were then removed, pipettes attached to the stumps, and the behavior of the plants was then observed for a few hours. The results for a group of six tomato plants are given in detail in table 1 and are shown graphically in figure 2 as algebraic averages of the six plants. The shoots were slightly wilted when removed, indicating that transpiration was probably exceeding absorption as usually occurs on bright days (Kramer, 1937). About one-half hour was required to attach the pipettes and begin measurement of exudation. During the first half hour of measurement water was absorbed through five of the six stumps. During the second half hour four root systems absorbed water and two exuded water, while during the third period three root systems showed exudation and three absorption. In the fourth half hour only one root system absorbed water, but it was not until the sixth period that water was being exuded from all six stumps. Later these root systems were attached to a vacuum pump, and the rate of water movement was found to be 3.5 times as great under a pressure gradient of 64 cm. of mercury as under atmospheric pressure.

The results of six experiments are summarized in table 2. Considerable difference exists between the behavior of the plants in the different experiments. Exudation reached almost its maximum in the first half hour in coleus, but not until the fourth half hour

in experiment two with tomato. In the experiments with hibiscus and balsam and the first experiment with tomato absorption occurred for a half hour to one and one-half hours after removing the shoots. Absorption ceased and exudation then began in hibiscus and tomato, but absorption continued in balsam until the soil was watered. These differences in behavior are evidently related to variations in soil moisture, rates of transpiration, and size of water deficit in the plants. In several experiments with tomato and sunflower it has been observed that if the tops of plants in moderately dry soil are removed, no exudation occurs until the soil is watered. This is sometimes true even though the shoots are not visibly wilted and the soil is certainly well above the wilt-

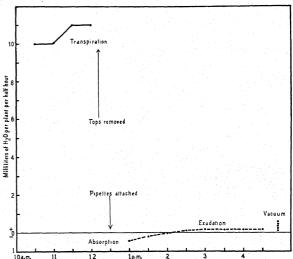


Fig. 2. Behavior of transpiring tomato plants following removal of tops. Note that the roots absorbed water through their stumps for over an hour after removal of the tops. The rate of water movement through the same root systems attached to a vacuum pump was 3.5 times the rate of exudation. The plants were growing in soil kept near its field capacity.

ing percentage. Probably the most significant fact to be obtained from table 2 is that exudation was always less than two per cent of transpiration.

Table 2. A comparison of transpiration and exudation. All rates are in ml. of  $H_2O$  per plant per half hour. The rates of exudation are for successive half-hour periods following removal of tops. The percentage relation is for the rate of transpiration during the last hour before removal of tops.

Number of		Trans	ranspiration			Exu	dation		140	Exuda- tion with	Exuda- tion as % of tran-
		1st hr.	2nd hr.	. 1	2	3	4	5	6	vacuum	spiration
Coleus 6		8.6	8.7	.16	.14	.17	.11			.30	1.5%
Hibiscus 5		5.8	6.7	n02	.01	.03	.02	.02	.04	• •	0.5%
Balsam 6		2.1	1.9	15	07	03	03 -	<b>—.</b> 03	03		
Sunflower 8		4.3	5.0	.01	.01	.01	.01	.01	.01	.08	0.2%
Tomato (1) 6		10.0	11.0	42	20	03	.10	.15	.16	.60	1.4%
Tomato (2) 6		7.5	8.7	.05	.09	.12	.15	.14	.15	.34	1.6%
				↑ Tops r	emove	d					

<sup>&</sup>lt;sup>a</sup> A minus signs means absorption of water by the stump instead of exudation from it.

Discussion.—The data presented here are representative of those obtained in experiments of this type. The rate of transpiration may vary widely with varying atmospheric and soil moisture conditions, while the rate of exudation seems to depend largely on past treatment and on soil moisture, aeration, and temperature. The general trend of these results is that which would be expected by physiologists familiar with this field of plant water relations. The principal new feature is the comparison of quantitative data on water intake of similar plants under several conditions.

The results obtained indicate that the rate of exudation from root systems of recently detopped plants amounts to only a small percentage of the water loss from the same or similar plants under conditions favorable to transpiration. If the rate of exudation is accepted as a measure of the rate of active absorption by a transpiring plant, then active absorption supplies a negligible amount of the water required to replace that lost in transpiration. It has been claimed, however, that we cannot evaluate the importance of active absorption in this manner because we do not know that it is proceeding at the same rate in a detopped plant as in an intact one. According to this view, the rate of active absorption or exudation of water into the xylem may have been much higher in an intact transpiring plant than it is in a root system from which the top has been removed. Consideration of the possible mechanisms which might be involved in active absorption gives no reasonable basis for such a belief. Investigators from Hales to the present writer have found that exudation frequently does not occur from the stumps for some time after removing the tops of freely transpiring plants. It seems highly improbable that if exudation or secretion of water into the xylem had been occurring during transpiration it would cease as soon as the top is removed, only to be resumed after an hour or two. It is the more doubtful since removal of the tops of slowly transpiring plants is immediately followed by exudation of water from the stumps.

The only possible method of measuring the rate of active absorption in intact plants seems to be by measuring the rate of guttation. Guttation is supposed to be caused by the same mechanism causing root pressure, and its occurrence, therefore, should be an indication of the occurrence of active absorption. Guttation occurs when conditions are favorable for absorption of water, but unfavorable for transpiration. If transpiration is stopped by placing a plant in a saturated atmosphere, guttation does not begin immediately. The behavior of guttation supports the view that active absorption may not occur in rapidly transpiring plants. While no quantitative comparisons are available, the volume of water exuding during guttation would amount to only a small percentage of the volume needed to replace that lost in transpiration.

It is often supposed that though active absorption may not be very important in transpiring plants, it does supplement physical absorption. It is probable, however, that little or no active absorption is occurring in the roots of many rapidly transpiring plants. The fact that the root systems of such plants often absorb water through the stumps for an hour or more after the tops are removed indicates that the root cells were not turgid when the tops were removed. It seems doubtful if any of the suggested mechanisms for secretion or osmotic movement of water into the xylem could function unless the cortical cells adjacent to the stele were turgid or at least had a diffusion pressure deficit lower than that of the contents of the xylem. If these cells were turgid, root systems of small plants in moist soil could not absorb one or two ml. of water through the surface of the stump after removal of the tops. Evidently the living cells of the roots absorb water from the xylem vessels, if it is supplied to the cut surfaces, and from the soil, if it is not too dry, until they are turgid. It is probable that only after the cells become turgid that active absorption can begin, resulting in movement of water into the xylem and its exudation from the open ends of the vessels at the stump. The writer has observed a number of instances in which no exudation occurred within 12 hours after removal of the tops, but then began within a few minutes when the soil was watered. This occurred with plants which were little or not at all wilted at the time of detopping and which were in soil certainly above the wilting percentage. This suggests that the maximum diffusion pressure deficit which can be developed in the roots of detopped plants may be lower than the maximum diffusion pressure deficit which can be developed in the roots of intact, transpiring plants. Kennedy and Crafts (1927) found that when the tops of rapidly transpiring morning-glory plants were removed, eosin and various toxic substances would often penetrate several feet into the roots and stain or kill them. The penetration was deepest and most rapid in dry soils and following periods of rapid transpiration. Penetration also occurred in roots of plants grown in water culture when exposed to the sun, but not if shaded prior to removing the shoots. It was suggested by Romell (1918) and by Köhnlein (1930) that the tension resulting from transpiration stimulates the root cells, causing increased passive absorption during periods of rapid transpiration. Köhnlein suggested this because his results indicated that the improbably high force of 20 to 73 atm. would be necessary to bring about the intake of sufficient water to supply a transpiring sunflower plant. His experimental procedure consisted of determining the transpiration rate of a plant, removing the top and attaching a vacuum pump, and determining the water intake with a given pressure gradient. From these data he calculated the root suction which must have been exerted to replace the water lost in transpiration.

In the experiment on which figure 2 is based the transpiration rate averaged 20 ml. per plant from 10 to 11 a.m. The rate of water movement through the root systems under a pressure gradient of 640 mm. of mercury averaged only 0.6 ml. per hour. On this basis a pressure gradient of about 28 atmospheres would have been required to supply the water needed to replace that lost in transpiration. Renner (1912a, 1929), using this and other methods, found somewhat lower forces necessary, but also decided that the values so obtained were improbably high and that the methods probably were unsatisfactory.

It is likely that the calculated pressure gradients were higher than necessary, because Brewig (1936) has shown that as the rate of transpiration increases the area of the root through which water is absorbed also increases. At low rates the absorbing zone is comparatively short, but as transpiration increases the tension in the vessels presumably also increases. This increases the steepness of the diffusion pressure deficit and pressure gradients, and the absorbing zone is extended toward the base of each root, greatly increasing the absorbing surface. This view is also supported by the work of Rosene (1937). It is probable, however, that fairly large pressure gradients are frequently developed across the cortex, since the cortical cells seem to offer considerable resistance to the movement of water (Kramer, 1938). Ordinarily a gradient of a few atmospheres probably exists, but if absorption lags much behind transpiration, a gradient of 100 atmospheres or more might possibly be developed in some plants (MacDougal, Overton, and Smith, 1929).

Consideration of the possible mechanisms bringing about active absorption and exudation into the xylem reveals no reason why any of the suggested mechanisms for active absorption would not function as well, as least for a few hours, in a detopped root system as in that of an intact plant. Whatever the exact mechanism, the necessary energy is probably supplied by the respiration of the cortical cells. This would simply require that they be supplied with sufficient food and oxygen to maintain normal physiological activities. That these conditions are fulfilled is probable, since a comparatively high rate of exudation is often maintained for several hours or even days after the tops are removed. The data of Grossenbacher (1938), for example, indicate that a comparatively high root pressure was maintained for at least a week by sunflower root systems, and Hoagland and Brover (1936) found that ion absorption by excised barley roots continued at a high rate for many hours.

Boonstra (1935) attempted to determine whether or not a positive pressure might exist in the roots of transpiring peas even though decreased pressure or tension existed in the stem. He sealed the plants into potometers in such a manner that any change in volume could be observed when the roots were severed from the stems. In some experiments part of the root projected above the stopper of the potometer so the lower part of the root was severed from the upper part. In every instance a sudden increase in absorption occurred immediately after cutting the roots, indicating that the pressure in the xylem of the roots was below atmospheric pressure rather than above, and that absorption rather than exudation had occurred through the cut surfaces.

The best way to determine the relative importance in water intake of the forces developed in the roots and those developed in the shoots probably is to separate the roots from the shoots. Renner (1912b) performed such experiments and decided that the absorbing power of the roots of transpiring plants is chiefly produced by the suction of the leaves. It was found by Kramer (1938) that the rate of absorption of transpiring sunflower and tomato shoots grown in water culture was significantly greater after removal of the root systems than it was with the root systems attached. Apparently instead of "pumping" water into the plant, the cells of the roots offered so much resistance to water movement that they were actually retarding absorption. This resistance to water movement is much greater at low temperatures.

It is known that exudation from the stump can often be stopped by placing the root system in salt or sugar solutions. Renner (1929) found that a reversal of water movement occurred in dilute solutions of sucrose and KNO<sub>3</sub>. The writer (1932) also found that exudation was stopped by dilute sucrose solutions. Tagawa (1934) carefully investigated the

relation between concentration of the surrounding solution and rate of water intake. He found that root systems of beans could not absorb water from sucrose solutions with an osmotic pressure of two atmospheres, but intact transpiring plants absorbed water from solutions with an osmotic value up to 14.6 atmospheres. He concluded that in these experiments the shoots developed an absorbing force of more than 12 atmospheres, the roots only 1.9 atmospheres.

A further reason for doubting that active absorption or root pressure phenomena play an essential rôle in the intake of water is the fact that it apparently never occurs in the gymnosperms. It would be very surprising if such a process were essential in one group of plants, yet not even occur in another group which is so similar in many respects. If we could learn why root pressure does not exist in gymnosperms, we might be better able to explain its oc-

currence in angiosperms.

It has sometimes been claimed that the metabolic activity of the root cells must be an important factor in the absorption of water because water intake is materially reduced by low temperatures and by poor aeration, both of which are known to reduce the rate of cell metabolism. It appears possible, however, that the physical effects of low temperature and poor aeration are sufficient, by themselves, to explain the decreased absorption of water. In experiments on root systems attached to a vacuum pump it was found that water intake through living roots at 2°C. was reduced to about 20 per cent of the rate at 25°C., and the rate of water intake through dead roots at 2°C. was about 50 per cent of the rate at 25°C. (Kramer, 1939). Low temperature increases the viscosity of water and decreases the permeability of the cell membranes, thus greatly increasing the resistance to water movement. This causes a large decrease in water intake at low temperatures which seems to be independent of the effects of low temperatures on cell metabolism. It has also been found that a high concentration of carbon dioxide causes a 30 to 50 per cent reduction in water intake through living root systems attached to a vacuum pump, a reduction which apparently may be ascribed to decreased permeability of the roots rather than decreased physiological activity of the root cells.

Although active absorption and root pressure apparently do not occur at all in some species and probably are not essential to any species, they may play a certain part in refilling xylem vessels with water which have become air-filled during periods of rapid transpiration. It is well known that the water content of many deciduous trees reaches a minimum during the late summer and gradually increases to a maximum in the autumn and winter (Gibbs, 1935). Perhaps this is more or less related to root pressure phenomena, though other explanations are possible. Seasonal variations in water content are less pronounced in conifers, possibly because they do not have root pressure.

While active absorption and root pressure are of common occurrence in many plants, it appears that

there is no conclusive evidence that they play either an essential or even an important part in the water relations of such plants. During times of rapid transpiration or low soil moisture, active absorption probably does not occur at all, and water intake is brought about by forces set in action by the loss of water in transpiration. At such times water may be said to be absorbed through the roots rather than by the roots. The occurrence of active absorption and the accompanying root pressure phenomena is possibly merely a fortuitous result of the root structure and of the manner in which salts are absorbed from the soil. Since it can only be studied in detopped plants, or possibly by observing guttation in entire plants, it is perhaps impossible to prove that active absorption or forces developed in the roots have no important rôle in the absorption of water. These experiments have shown, however, that the forces which have been measured and discussed by earlier workers as root pressure or exudation phenomena are wholly inadequate to supply the water requirements of a transpiring plant.

#### SUMMARY

The relative importance of passive absorption caused by forces produced in the transpiring shoots and of active absorption caused by the osmotic or secretory activity of the root cells has often been debated. A quantitative comparison of the amounts of water entering plants by active and by passive absorption was therefore made in order to better evaluate the relative importance of the two mechanisms.

A comparison was made of the rates of transpiration of potted coleus, hibiscus, balsam, sunflower, and tomato plants with the rates of exudation from the stumps of the same or similar plants following removal of the shoots. The rate of water movement through detopped root systems attached to a vacuum pump was also determined.

It was found that the rate of exudation from detopped root systems was only one to five per cent of the rate of transpiration from similar intact plants.

When the tops were removed from freely transpiring plants, frequently no exudation occurred, but instead water was at first absorbed through the freshly cut stem surfaces, and exudation began only after one-half to two hours. In some experiments exudation did not begin until the soil was watered.

Several previous investigations have shown that intact plants can absorb water from much more concentrated solutions than can detopped root systems. This seems to indicate that the greater part of the forces causing the intake of water originate in the transpiring shoots rather than in the living cells of the roots.

Root pressure has never been observed in the gymnosperms, and it is probable that active absorption never occurs in that group. It seems doubtful if a process which never occurs in one division of the seed plants would be essential in another group which is otherwise quite similar physiologically.

It is concluded that active absorption, as made evident by exudation and root pressure phenomena,

is wholly inadequate to supply the water requirements of transpiring plants. It is possible that active absorption does not even supplement passive absorption in rapidly transpiring plants, because none of the suggested mechanisms for physiological absorption would operate unless the cells of the roots are turgid. Possibly root pressure is a fortuitous result of the root structure and of the manner in which minerals are absorbed by certain plants.

Most, and possibly under some conditions all, of the water absorbed by transpiring plants is absorbed as a result of forces set in motion by the loss of water in transpiration. Removal of water in transpiration decreases the pressure or causes tension on the water in the xylem, producing a gradient of decreasing pressure and increasing diffusion pressure deficits along which water moves by mass flow and to some extent by diffusion from the epidermis to xylem. The roots act as passive absorbing surfaces in such a mechanism, but healthy growing root systems are very important because extension of roots through the soil continually makes available new supplies of moisture.

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# DIFFERENTIAL GROWTH IN PLANT TISSUES. II. A MODIFIED AUXIN TEST OF HIGH SENSITIVITY 1

Kenneth V. Thimann and Charles L. Schneider

When elongating stems or other organs are slit longitudinally and placed in auxin solutions, the halves curve toward one another, as was first shown by Went (1934). In the course of an analysis of this and similar reactions, it was pointed out (Thimann and Schneider, 1938) that Avena coleoptiles are among the large number of objects which respond well. The present paper will describe a modification of the procedure, using Avena coleoptiles, which enables auxin to be quantitatively determined at very much lower concentrations than has previously been possible. Certain of the theoretical implications of this reaction will also be considered.

THE "QUARTERED COLEOPTILE" TEST.—Procedure.—Avena coleoptiles, from plants about 3 cm. long grown in the dark-room on wet filter-paper with their roots in tap water, are decapitated at 3 mm. from the tip, slit longitudinally downward for a distance of 2 cm., turned through 90°, and again carefully slit downward for the same distance. We have also used a special cutter (fig. 1), consisting of

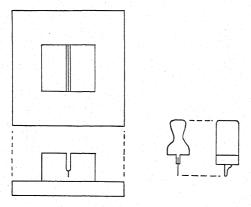


Fig. 1. Tool for quartering coleoptiles. Top and front view of holder and front and side view of cutter. See

CUTTER

HOLDER

text.

two pieces of brass, one a holder with a groove 1.5 mm. wide (in the bottom of which the coleoptile is laid), the other a cutter consisting of a brass handle with a guide 1 cm. long which just fits into the groove and which has inserted into it a fragment of razorblade. This blade is centered during the cutting operation by means of a very fine slit (about 0.2 mm. wide) at the base of the groove; in this slit the blade just slides freely without touching the bottom. The intact coleoptile, with leaf in, is slit for a distance of 1.5 cm., terminating a few mm. below the tip, then

<sup>1</sup> Received for publication September 16, 1939. <sup>2</sup> Made for us by Mr. J. A. Westgard of these laboratories. turned through 90° and slit again. The tip and leaf are subsequently removed. The resulting quartered coleoptiles are placed in a Petri dish of the solution to be tested. After 24 hours at 25° in the darkroom the curvatures are estimated to the nearest 10° either with a 360° protractor, or (with a little practice) by eye, and the mean taken. In distilled water the quarters curve outward through a very large angle—of the order of 250°, though varying somewhat from test to test. In dilute auxin solutions this outward curvature is reduced, and in higher concentrations the curvature becomes inward.

Sensitivity.—In the older procedure the coleoptiles were slit only once—i.e., halved. If we compare the curvatures obtained by quartering with those obtained by halving, a marked difference is observed. In indole-acetic acid, the halved coleoptiles show a definite decrease in outward curvature (the first step toward inward curvature) at 0.1 mg. per liter. This is also exemplified in figure 3 of Thiman and Schneider (1938). With the quartered coleoptiles, however, the very large outward curvature in water is slightly increased at about 0.00002 mg. per liter and shows decrease at concentrations of 0.0003 mg. per liter and above. In both methods

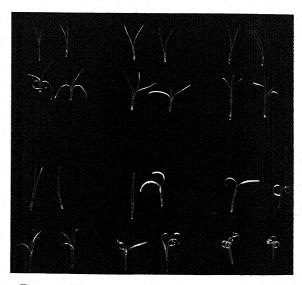


Fig. 2. Halved and quartered Avena coleoptiles after 24 hours in distilled-water auxin solutions of strengths: left to right, top, 0, 0.0016, 0.04; bottom, 0.2, 1, and 10 mg. per liter.

the concentration causing actual inward curvature is about the same.

The responses of halved and quartered coleoptiles are illustrated in figure 2 and shown quantitatively in figure 3, which is a mean of three complete experiments with indole-acetic acid.

As will be noted, the curve shows an inflection at about 0.05 mg. per liter. In some of the many experiments performed this inflection was still more marked, in others (e.g., fig. 5), it was practically absent. Such an inflection might suggest that two different effects are present—one causing actual inward curvature, which is essentially the same as with halved coleoptiles, and another causing decrease in the large outward curvature, which is completely missing from the responses of the halved plants. However, not only is there no direct evidence that the two effects are really different, but the comparison of different auxins (see below), and the considerations advanced in the discussion, strongly indicate that reduction in outward curvature and appearance of inward curvature are both part of the same phenomenon and merge continuously into each other.

In the standard Avena test, using agar blocks, the lowest concentration giving practically measurable curvature is about 0.01 mg. indole-acetic acid per liter of solution, which when mixed with an equal

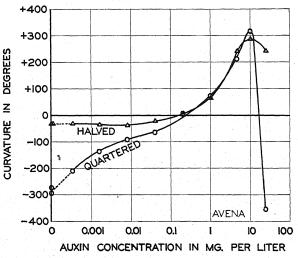


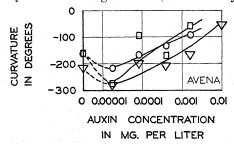
Fig. 3. Comparison of the curvatures of halved and quartered Avena coleoptiles in serial auxin dilutions.

volume of 3 per cent agar gives about 2°. With the deseeded test of Skoog (1937) or with Cephalaria seedlings (Söding, 1936), this lowest concentration may perhaps be decreased by as much as five times. The modified slit stem test on peas, described by Went (1939), responds to about the same concentrations as the standard Avena test. Thus the quartered coleoptile test gives easily measurable curvatures, say 50° less than that of the controls, at concentrations 30 times lower than those effective in the standard test and some six times lower than those effective in the deseeded and Cephalaria tests.

The suggestion may be advanced that the lowest effective concentration approaches the true relationship between the auxin and its substrate in the cell. In the case of decreased outward curvature, which is a response of the outermost tissue layers, it is a

simple matter to calculate the amount of auxin needed per cell. The average volume of the epidermal cell of the coleoptile is about 10<sup>-4</sup> mm.<sup>3</sup>, so that (assuming equal distribution of auxin inside and outside the cell) at a concentration of 3·10<sup>-4</sup> mg. per liter we have 3·10<sup>-14</sup> mg. auxin, or about 90,000 molecules, per cell.

The increase in outward curvature, due presumably to increased growth of the inner layers (see



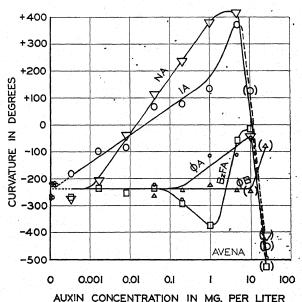


Fig. 4 (above). Curvatures of quartered Avena coleoptiles in very dilute auxin solutions. The three curves are

three separate experiments.

Fig. 5 (below). Curvatures of quartered Avena coleoptiles in serial dilutions of several synthetic auxins. IA, indole-3-acetic; NA,  $\alpha$ -naphthalene-acetic;  $\Phi$ A, phenylacetic; BzFA, benzofurane-3-acetic;  $\Phi$ B,  $\gamma$ -phenyl-butyric acids.

Thimann and Schneider, 1938), appears at still lower concentrations. In order to be certain of its reality a number of experiments were carried out with very low concentrations; these are summarized in figure 4. It will be seen that the inner layers respond to concentrations around 0.00001 mg. per liter, or one part in 10<sup>11</sup>, an excessively low figure. It is of interest to note that this corresponds with the concentrations found to promote root growth (cf. for instance Geiger-Huber and Burlet, 1936). If we assume that the cells do not accumulate auxin

and that their volume is the same as that of the epidermal cells, the minimal amount for a measurable effect would be 3000 molecules of auxin per cell.

Variation in results.—Most of the sources of error lie in the slitting process. Variation in length of the slit and in width of the quarters is hard to avoid. Twisting of the quarters may also result from confinement to the shallow layer of solution, but this can be easily detected and allowed for. The variation within one test can, of course, be treated statistically. Using four plants (16 curvatures) the probable error of the mean in the water controls is generally 10°-12°. If the probable error of the mean in the lowest auxin concentration is the same, then the probable error of the difference between the two is  $\pm \sqrt{12^2 + 12^2}$  or about  $\pm 17^{\circ}$ . In other words, to show minimal auxin concentration, the outward curvature should be reduced by 50°. This requires a concentration of 0.0003 mg. indole-acetic acid per liter (see figures 3 and 5).

Influence of vascular tissues.—Quartered coleoptiles in water or very low auxin concentrations frequently show two segments with strong outward curvatures (averaging 250°) and two with weak curvatures (averaging 150°). An example may be seen in the water controls of figure 2. In such cases it is invariably found that the vascular bundles are in the strongly curved segments (cf. fig. 6B). In a long series of tests with different concentrations of indole-acetic acid ranging from 0.00001 to 10 mg. per liter, the segments with the vascular bundles curved outward about 100° more, or inward about 100° less, than the others.

That the effect is not due to pressure against the bottom of the dish in the shallow layer of liquid may be shown by using very deep layers of aerated water, in which the bases of the quartered coleoptiles are fixed to glass pins, the whole coleoptile being thus immersed and the quarters free from the walls. The marked difference persists under these conditions.

The explanation of this phenomenon is probably very complex and will not be attempted here. The data in this paper are the averages of the "vascular" and "non-vascular" quarters throughout. Since the differences between these two are about 100° and show no trend with changing auxin concentration, the curves of concentration against curvature merely parallel one another.

Other influences.—The outward curvature of water controls is considerably smaller when tap water is used. In one experiment the controls in dis-

tilled water gave 217°, those in tap water 80°; in another experiment the values were 348° and 138°, respectively. Similarly, old distilled water which had been kept in soft glass carboys for about six weeks gave outward curvatures some 60° lower than those in fresh distilled water. It appears that the coleoptiles are sensitive to traces of some salt or salts.

This salt effect was tested over the whole range of auxin concentrations. In table I curvatures in solutions made up with distilled water are compared with those in solutions made up with tap water. The effect of the tap water decreases somewhat as the auxin concentration increases, although it is present throughout the range. The differences between curvature in auxin and curvature in the water controls, however, are consistently smaller when tap water is used

The outward curvature is also decreased when very small volumes of solution are used. In experiments with four coleoptiles in 20 cc. of distilled water, the curvatures were 293° and 324° (cf. data in fig. 3); with two coleoptiles in only 2 cc. in the same experiments, the values were 116° and 205° only. This effect has been observed on numerous occasions. It was first thought that certain salts, or possibly auxin itself, might have diffused into the water from the cut surfaces. To test this, two coleoptiles were placed in each of ten 2 cc. samples of distilled water for 24 hours; these samples of water were then combined to make one quantity of 20 cc., into which fresh coleoptiles were placed. The resulting curvatures were slightly, but not significantly, lower than those given in a new sample of distilled water. In another experiment the water in which quartered coleoptiles had been placed was extracted with ether and the extract tested for auxin content, but with completely negative results. Aeration of the 2 cc. volume also did not increase the curvature. It is thus difficult to ascribe the effect either to material diffusing from the coleoptiles or to oxygen deficiency. Whatever be the reason, this effect constitutes a limitation on the application of the quartered test to small volumes, since with the smaller outward curvature of the controls the sensitivity to the lowest auxin concentrations is somewhat reduced (see the experiment below).

Some applications of the method.—Comparison of different auxins.—The results obtained using serial dilutions of a number of auxins are shown in figure 5. The principal points to be noticed are: (1)

Table 1. Comparison of auxin curvatures of quartered coleoptiles in distilled water and tap water solutions. A plus sign indicates inward curvature; a minus, outward.

Concentration of indole-acetic acid, mg. per liter:	0	0.0002	0.001	0.01	0.1	1.0	10.0
	11 11 11	Cu	rvature	(mean o	f 16 value	s)	
Distilled water solutions Actual curvature  Difference from control			288 62	67 283	—36 314	+199 549	+605 955
Tap water solutions Actual curvatures  Difference from control			—72 49	+12 133	+110 231	+304 $425$	+711 832

Table 2. Relative activities as determined by the quartered coleoptile test compared with those determined by other Avena methods.

	Quartered coleoptile test	Straight growth test	Standard Avena test (agar-curva- ture)
Indole-3-acetic acid	. 100	100	100
$\alpha$ -naphthalene-acetic acid	. 35	15	2.5
Phenyl-acetic acid		0.3	0.02
Benzofurane-3-acetic acid	. 0.03	0.1	0.02
γ-phenyl-butyric acid		0.06	0.005

indole-acetic acid is active at lower concentrations that any other compound tested (the concentrations giving outward curvatures were not included in these experiments), (2) the curve for a-naphthalene-acetic acid has a steeper slope than that for indole-acetic, and (3) benzofurane-acetic acid gives large outward curvatures at concentrations around 1 mg. per liter, which change to inward curvatures at about 5 mg. per liter. All these observations agree with those previously obtained with the slit pea-stem test (Thimann and Schneider, 1939), which suggests that they represent phenomena of general importance.

For each auxin studied, the range of concentrations causing inward curvature is continuous with that reducing the outward curvature. There is therefore no reason here to suppose that decrease in outward curvature is anything but the first step toward inward curvature.

The principal difference from the test with peastems lies in the much greater selectivity of the Avena coleoptiles-i.e., at low concentrations the differences between different auxins are relatively great. If as measure of activity we take the reciprocal of that concentration which reduces the outward curvature by 100°, the relative activities of the different auxins can be assessed. These values, expressed as percentage of the activity of indoleacetic acid, are listed in table 2, together with values obtained from other methods previously reported. It will be seen that the quartered test is about as selective as the other Avena methods. As already pointed out, a few comparative tests by several different methods, using a series of concentrations of different auxins, would serve to identify, with a fair degree of certainty, as unknown auxin with a known one.

Botanical tests.—The sensitivity of the quartered coleoptile to very dilute auxin solutions suggests its application to numerous problems of auxin distribution. Two examples may be given.

From the data in the literature on the production of auxin by coleoptile tips, it may be calculated that one tip, after cutting off, should set free into 2 cc. of solution a total amount of auxin roughly equivalent to 0.0002 mg. indole-acetic acid per liter. Hence two to four tips, placed in 2 cc. of water, should give enough auxin to produce significant curvature in the quartered test.

An attempt was therefore made to use the test to detect the auxin diffusing from a few coleoptile tips. Four quartered coleoptiles were placed in 2 cc. of water into which freshly cut *Avena* tips were introduced. Table 3 gives the mean of three such experi-

Table 3. Measurement of auxin production by Avena coleoptile tips and by Phaseolus nodules using the quartered Avena coleoptile test as assay method.

No. of tips or half-nodules:	0	2	4	8	16	32
Outward curvature for:						7
Coleoptile tips	116	111	129	54	55	44
Root nodules			133			

ments. A significant decrease of curvature is produced when eight or more tips are used. This effect can hardly be due to traces of salt diffusing from the tips, since far more of such material would be provided by the four quartered coleoptiles, with their long cut surfaces, than by the tips. The outward curvature of the water controls in these experiments is in any event already lowered by the volume effect mentioned above, so that the further lowering can safely be ascribed to the auxin coming from the tips. It is, indeed, because the water controls give curvatures lower than normal that two or four tips do not give the expected effect. However, it is satisfactory that the small amount of auxin produced even by eight tips when dispersed in 2 cc. of water should be so readily detectable.

A comparable experiment was made with root nodules of *Phaseolus vulgaris*. Nodules about 2 mm. in diameter were halved and varying numbers placed in 20 cc. of water. As table 3 shows, water in which four nodules were placed reduced the outward curvature by 63°, which is fully significant. Sixteen nodules (32 halves) are even more active than 32 tips. It follows that these nodules are at least as effective in auxin production as the coleoptile tip.

On the other hand, soil solution, made by shaking 80 grams of garden soil with 100 cc. of tap water and filtering, gave curvatures no different from those of tap water controls. The auxin content of such soil solution must therefore be extremely low.

MECHANISM OF THE CURVATURE.—Although it is now generally agreed that curvatures of the type dealt with here are caused by differences between the amounts of growth of which the inner and outer layers of the slit object are capable, it is not yet certain to what this difference in growth is due. According to the view previously put forward (Thimann and Schneider, 1938), the difference in growth is due to an inherent difference in sensitivity between the inner and outer tissues, while according to Went (1939) it is the result of the wounding process itself. Our own experiments are in agreement with those of Went, and also of van Overbeek and Went (1937), in showing that wounding does somewhat decrease the response of stem tissues to auxin. The effect of the wounding may therefore contribute to the cur-



Fig. 6. Three methods for slitting coleoptiles. A, halving; B, quartering; C, narrow strips with cuts on two of the strips at right angles to the plane of curvature. See text.

vature, but, as will be shown, it is improbable that it is an essential for it.

In the first place, Avena coleoptiles are hollow, so that when they are slit they have only a very small area of wounded surface. Yet they give inward curvatures of about the same magnitude and sensitivity as do pea stems, which have a much larger area of wounded tissue.

In the second place it must be remembered that inward curvature (i.e., bending toward the wound) is not the only phenomenon observed. Low concentrations of both indole-acetic and a-naphthaleneacetic acids cause increased outward curvature, and with benzofurane-3-acetic acid, from 0.2 to about 2 mg. per liter, this effect is extremely strong. Here the wounded side must be growing more than the outer intact side. It must be noted that these outward curvatures do not differ in their time relations from inward curvatures. This is shown clearly in figure 11 of Thimann and Schneider (1938) in which the outward curvature in 2 mg. per liter benzofurane-acetic acid takes about 15 hours, while inward curvature in I mg. per liter indole-acetic acid takes about 18 hours. Thus all the evidence is consistent with the view that this increased outward curvature is a true growth response.

The present experiments provide additional evidence as to the mechanism of the curvature. As the diagram, figure 6B, shows, the wounded surfaces of quartered coleoptiles are at an angle to one another, so that the influence which they can exert on growth is limited to that component in which they reinforce each other. In spite of this fact the magnitude of the curvatures is just as great as with the halved coleoptiles, while the sensitivity to low auxin concentrations is, of course, much greater.

In order to minimize the effect of wounding, coleoptiles were quartered so as to obtain strips having the cut surfaces parallel and thus with no component in the direction of the curving (see figure 6C). Such strips regularly curved inward in auxin of concentrations from 0.04 to 5 mg. per liter. To make such cuts with accuracy, however, is difficult on Avena, in which the coleoptile is only 1.2 mm. in diameter. More striking results were obtained with Zea Mays coleoptiles, which are much larger. In figure 7 camera lucida drawings of three such curvatures are shown. The sections show strong inward curvatures, although the two wounded surfaces remain almost parallel. These experiments confirm the view that the curvatures are essentially due to inherent differences in the response to auxin of inner and outer tissues, or, as Jost (1938) has expressed it, that the curvature is nastic rather than tropistic. The conclusions from Jost's experiments are in full agreement with those to be drawn from our own.

Some other points call for mention here. One arises from consideration of the geometry of a half-or quarter-cylinder such as a slit coleoptile. This

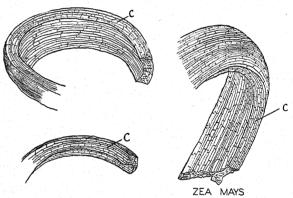


Fig. 7. Camera lucida drawings of strips of Zea Mays coleoptiles curving in auxin solution. The injured surfaces, C, have little or no component in the plane of curvature.

may be regarded as a trough or groove, and evidently the degree of longitudinal curvature will not be without effect on the degree of transverse curvature or "grooving." When rubber tubing is slit longitudinally and then bent, the groove will tend to become flattened irrespective of whether the curvature is "inward" or "outward." This flattening may be readily observed in outwardly curving slit coleoptiles, but it does not happen when the curvature is inward. Instead, the "grooving" generally becomes even deeper—i.e., the segment of tissue actively curves inward in cross section as well as in length. This indicates that auxin has some influence in promoting tangential as well as longitudinal growth and that this influence is different on the inner and outer tis-

<sup>3</sup> A similar experiment is reported by Went (1939), but he did not obtain inward curvature. In a number of trials our only failures have been when the strips were extremely narrow. In such very narrow strips the proportion of cells adjacent to wounded surfaces is very high, and thus the "damage effect" may perhaps become important enough to prevent much growth. However, other explanations are not excluded.

sues. It must be emphasized, however, that the increase in "grooving" is a relatively small effect and is not itself responsible for the curvature.

The difference between the behavior of the quartered and halved coleoptiles is evidently largely a mechanical one, because pea stems also show it. In figure 8 are compared the curvatures of etiolated Pisum internodes prepared by the two methods. The quartered sections show larger outward curvatures in water than do the halves, while the lowest auxin concentration which gives a significant decrease in outward curvature is at least ten times lower than with the halved sections. Evidently, as with Avena, when the material is only halved, the left-hand end of the curve is completely lost, and this is almost certainly due to the mechanical stresses inherent in the differently shaped cross section. For this reason it seems improbable that the lowest concentrations active in the halved test have any "stoichiometrical" significance.

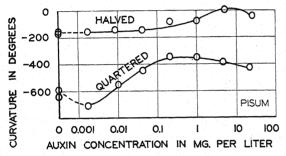


Fig. 8. Curvature of halved and quartered *Pisum* stems in serial auxin dilutions.

The same difference between the behavior of quartered and halved coleoptiles may be seen in the curvatures caused by acid. As is well known, acid solutions greatly increase the outward curvature. Figure 9 shows that here also the sensitivity to low concentrations of acid is greater with the quartered material. It may be noted that if the explanation of the acid curvatures given by D. Bonner (1938) is correct—i.e., that they are due to a setting free of the undissociated form of the auxin (i.e., the free acid) on the cut side—then it follows that the cut

side must have retained its ability to grow in response to the auxin set free. Bonner's calculations do not indicate a very great increase in the proportion of undissociated auxin on the cut side, yet the acid curvatures are very large. This also would not

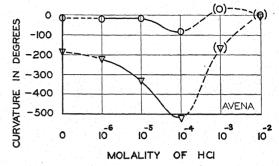


Fig. 9. Curvatures of halved (circles) and quartered (triangles) Avena coleoptiles in serial dilutions of HCl.

support the view that the wounded side has lost its sensitivity to auxin.

### SUMMARY

An assay method is described in which Avena coleoptiles are slit into four parts longitudinally and these slices allowed to curve in auxin solutions. The test is some thirty times as sensitive as the Avena test with agar and can detect concentrations of indole-acetic acid as low as 0.01 gamma per liter.

Various factors affecting the test are discussed, and some examples of its use for dilute auxin solutions and for comparison of different auxins are given.

It is pointed out that the increased sensitivity of the test is probably mainly to be ascribed to mechanical factors affecting the curving sections.

The essential features of the test support the view that curvatures of this type are due to inherent differences in the response to auxin of adjacent layers of tissue.

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# TWO OCCURRENCES OF NATURAL PAPER FORMED BY ALGAE <sup>1</sup>

S. C. Bausor

RECENTLY MORELAND (1937) described an instance of the formation of natural paper by the matting and drying of algal filaments, mostly Tribonema, in fields inundated by the overflowing of the Atchafalaya and Ouchita Rivers in Louisiana. This spring two similar cases of the formation of natural paper from algal remains were discovered in temporary marshes, and in both instances the paper was made up predominantly of the alga Tribonema.<sup>2</sup>

On May 7, at the intersection of Crystal Street and Harrison Avenue in Harrison, New York, I came upon a swamp in which *Tribonema* was grow-

Another alga of filamentous organization was found in the marsh and also was incorporated into the paper. It was a much smaller filament  $(37~\mu \times 5~\mu)$  with relatively thick walls which were Tribonema-like. The dried paper was white with a grayish cast and rather soft to the touch when relatively free of débris. This swamp was revisited on June 3. It had dried up completely, and the whole area previously under water was now covered with a mat of algal paper (fig. 1).

On May 21, the writer visited a rather large temporary swamp in the Pocono Mountains near Analo-

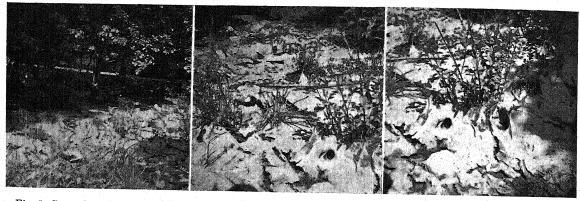


Fig. 1. General view of a portion of the dried up marsh at Harrison, New York, June 3 (left); paper covering the floor of the marsh (center); and a close-up of the algal paper on the floor and draping stubble (right).

ing luxuriantly. The swamp was drying up rather rapidly, and the algae which were left stranded along the edge of the marsh dried in paper-thin mats on the fallen leaves and other débris on the floor of the swamp. Twigs and stubble sticking up in the still-submerged portions caught masses of the algae as the level of the water became lower. Those exposed were in process of drying into sheets of paper.

Examination of the living algae and the paper showed conclusively that Tribonema bombycinum (Ag.) Derb. & Sol. was the predominant filamentous (fibrous) material in both. When treated with hot concentrated hydrochloric acid, the filaments of the algae and the fibers of the paper became separated into the H-shaped segments characteristic of Tribonema. When the algae and the paper were treated with a saturated solution of potassium hydroxide, the stratification and interlocking of the two portions of the wall became evident as the wall material swelled. The cells of Tribonema bombycinum averaged 63.3  $\mu$  in length and 13.3  $\mu$  in diameter, while the cells of the fibers in the paper after soaking were 51.8  $\mu$  in length and 14.0  $\mu$  in diameter.

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<sup>2</sup> Specimens of the paper and the algae are deposited at the New York Botanical Garden and at the Field Museum of Natural History, Chicago. mink, Pennsylvania, in which natural paper was forming. This marsh was similar to the one at Harrison. Microscopic examination showed that the algal mass and the paper were predominantly Tribonema bombycinum. In addition, however, other algae of filamentous organization were found mingled with the Tribonema in both the paper and the living algae. Among the more abundant of these were a species of Zygnema, an unidentified filament containing aplanospores, and the blue-green alga Anabaena.

The formation of natural paper by the matting of interwoven fibers of *Tribonema* apparently is not an uncommon phenomenon and probably may be expected wherever a stagnant body of temporary water supporting a luxuriant growth of algae is subjected to rapid evaporation.

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<sup>8</sup> Dr. Harold C. Bold kindly identified this as *Tribonema bombycinum* forma *tenue* Hazen.

# ULTRA-VIOLET SPECTROPHOTOMETRY OF ZEA MAYS POLLEN WITH THE QUARTZ MICROSCOPE <sup>1</sup>

Fred M. Uber

QUANTITATIVE INTERPRETATIONS of data on the biological effects of ultra-violet radiation must usually be based on a knowledge of absorption by the organism involved. Information of this kind has been used most often in an attempt to correlate biological effects directly with the total energy absorbed. Less frequently, the important question of a screening effect has been the desideratum. Owing to the slight penetration of living matter by ultra-violet, one is ordinarily concerned with objects of microscopical size, at least in one dimension. Where the organisms are such as to be compressible into thin uniform layers, data can be obtained rather readily, as in the bacterial measurements of Gates (1930). Where single organisms must be subjected to measurement, the use of a microscope is indicated. In the latter category are the investigations by Vles and Gex (1934) on sea urchin eggs, by Baas-Becking and Ross (1925) and Albers and Knorr (1937) on chloroplasts, and the splendid work of Caspersson on the salivary gland chromosomes of Drosophila. Ultraviolet absorption spectra of microscopic preparations have also been studied by Dubouloz (1938), Prat (1936), and Lucas (1934).

Ultra-violet transmission data on suspensions of organisms have been reported by Ehrismann and Noethling (1932) for bacteria and yeast, by Noethling and Stubbe (1934) for Antirrhinum pollen, and by Giese and Leighton (1935) for paramoecia. This method is far from satisfactory owing to the scattering by the organisms and the difficulties of secur-

ing data of absolute value.

The present investigation on the absorption characteristics of corn pollen has grown out of a need for accurate information as to the actual energy reaching the chromosomes within a pollen grain in order to interpret genetic results (Stadler and Uber, 1938). No existing data on the composition of pollen, either of walls or contents, are sufficient to solve this problem on the basis of known absorption curves of the chemical constituents.

The writer wishes to record his appreciation of the technical assistance of Mr. Roger Winters and Dr. Ernest W. Landen, and of the privilege of frequent consultation with Professor L. J. Stadler. The quartz microscope was made available by a generous grant of the University of Missouri Research Council.

Theory of absorption measurements with the microscope.—When a parallel beam of radiation is absorbed in a homogeneous layer of substance of uniform thickness, D, the relation between the incident flux,  $I_0$ , and the transmitted flux, I, can be expressed by the equation:  $I = I_0 e^{-kD}$ , where k is the extinction coefficient for the substance and e is the nape-

<sup>1</sup> Received for publication August 11, 1939.

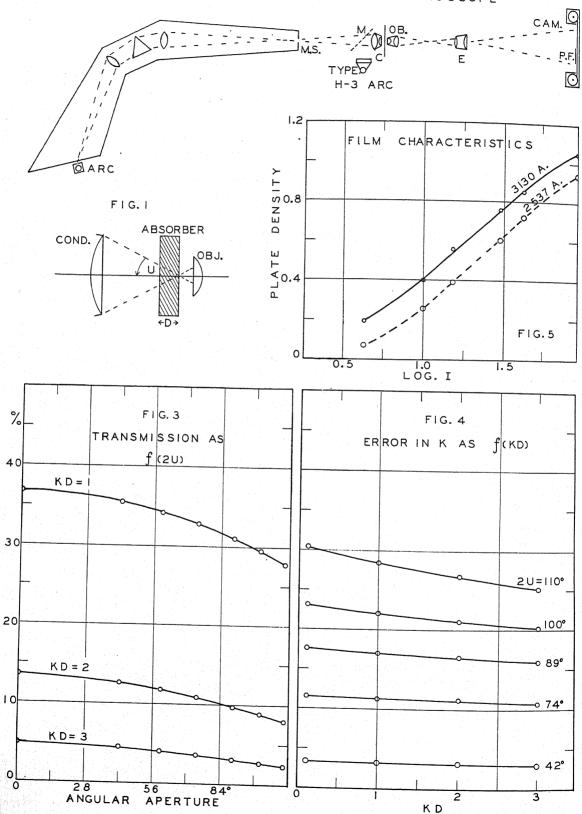
rian logarithm base. Accurate determinations with the microscope of the extinction coefficients, k, for a substance at various wave lengths, will be seen to depend on a knowledge of several factors, which will now be discussed in some detail.

Since energy measurements are made on the image formed by the microscope, it is essential that there be exact correspondence between radiation intensities at the image and at the object. For objects which are large compared to the limit of resolution of the microscope, this condition of correspondence depends only on the degree of optical perfection of the microscope. In other words, the image must be a perfect replica of the object. The most important corrections of the lenses in an ultra-violet microscope are for spherical aberration, coma, and curvature of the field, since chromatic aberrations do not enter where only monochromatic radiation is used. Compensation for the variations in spherical aberration with wave length can be effected by alterations in tube length. Quartz objectives as developed by Köhler (1904) are unusually well corrected for the aberrations in question. A chromatic correction, though highly desirable for spectrophotometric investigations, is practically impossible owing to a lack of suitable optical materials.

Several properties of an object under investigation may influence the determination of absorption data—namely, its size, shape, refractive index, and degree of homogeneity. The case where the object is large compared to the limit of resolution of a microscope has already been mentioned and is the one with which the present study is concerned. Where the dimensions of an object are of the same order of magnitude as the limit of resolution, the intensity ratios in the image no longer correspond to those in the object; indeed the shape of the image may bear little or no resemblance to the shape of the object. Hence, absorption measurements become very difficult or even impossible. This case has been discussed at some length by Caspersson (1936), who has also worked out the consequences of shape for absorption determinations as a function of the refractive index of the object. Caspersson concludes that an object must have dimensions approximately four times the wave length of the radiation used in order for its image to simulate the object well enough for absorption measurements.

Where a convergent beam of radiation is transmitted through an absorbing layer as depicted in figure 1, various parts of the beam traverse different distances of path within the layer. Hence, one cannot employ simply the thickness, D, of the layer in the equation to obtain the extinction coefficient. Instead of the transmitted flux through the absorbing substance and the control, I and  $I_0$ , respectively, being

FIG. 2 MONOCHROMATOR AND QUARTZ MICROSCOPE



related by the equation  $I = I_0 e^{-kD}$ , one finds, as did Caspersson (1936), the following ratio effective:

$$\frac{I}{I_o} = \frac{\int_o^u \sin u \ e^{-kD/\cos u} \ du}{\int_o^u \sin u \ du}$$

where 2u is the angle subtended at the object by the objective or by the condenser, whichever is the smaller. Upon integrating this expression, one obtains the following infinite series:

increased. In practice this need not be the case if there is appreciable scattering by the object, for the greater the aperture the larger the quantity of scattered radiation which will enter the objective. As will be seen from figure 9, the transmission of pollen walls as measured with a 2.5 mm. objective is greater than the value for a 6.0 mm. objective. Some idea of the amount of scattered radiation can be obtained in this manner, but to be quantitative one would

The following infinite series:
$$\frac{I}{I_o} = \frac{\left[ -e^{-kD/\cos u} \cos u - kD \left( \log \sec u - \frac{kD \sec u}{1 \cdot 1!} + \frac{(kD \sec u)^2}{2 \cdot 2!} - \frac{(kD \sec u)^3}{3 \cdot 3!} + \ldots \right) \right]_0^u}{1 - \cos u}$$

This equation can be evaluated either directly or by graphical integration for the various apertures which are ordinarily used in the quartz microscope, assuming definite values for kD. The ratios of  $I/I_0$ for various angular apertures can be compared with the ratio of  $I/I_0$  which would be given for a parallel beam (0 angle) by referring to the curves in figure 3. The curves show the percentage transmission for kD equal to 1, 2, or 3. The percentage error in the extinction coefficient as a function of kD for various angular apertures is shown in figure 4. It will be seen that the error in k when using the 6.0 mm. dry quartz objective with an angular aperture of 42° is less than 4 per cent and that this error in k is fairly constant with respect to kD, decreasing from 3.5 to 3.1 per cent as kD increases from 0.1 to 3.0, which corresponds to a change in transmission from 90 to 4.5 per cent. For the 2.5 mm. glycerine-immersion objective with an angular aperture of 90 degrees, the error in k is around 17 per cent, while the 1.2 N.A. immersion objective (2u = 110°) would result in an uncorrected value of k about 25-30 per cent too high.

Since k is proportional to the cologarithm of the transmission  $I/I_o$ , it is obvious that the variations in transmission of an absorbing layer with aperture must be greater than those just given for k. The transmission as measured with the 1.2 N.A. objective is around 50 per cent of that for a parallel beam when kD = 3, 60 per cent when kD = 2, and 75 per cent when kD = 1. For the 6.0 mm. objective, the corresponding transmissions range from 90 to 96 per cent and for the 2.5 mm. immersion objective, from 71 to 84 per cent, respectively, of the values obtained by using parallel radiation.

According to the above theory, one would anticipate a decrease in transmission as the aperture is

have to know the distribution of scattered radiation as a function of the angle of scattering.

Apparatus and technic.—The ultra-violet microscope.—The arrangement of the quartz microscope with reference to the monochromator and accessory equipment is shown in figure 2. The horizontal disposition proved highly satisfactory, a stable optical bench serving as a base upon which each part of the microscope could be adjusted independently.

The optical equipment consisted of the standard Zeiss quartz condenser, the 5, 7, 10, and  $14 \times$  eyepieces, and the 6.0 mm., 2.5 mm., and 1.7 mm. objectives, the last two objectives being for glycerine immersion. These have been amply described by Köhler (1904). Although corrected for the wave length 2750 Å, the objectives can be used satisfactorily over a range of several hundred ångstroms, particularly where finest detail is not a consideration. Moreover, the slight alterations of the corrections with wave length can be obviated by proper changes in the tube length of the microscope, should it prove important. As an immersion fluid, glycerine-sucrose solutions were employed.

Focusing was accomplished in the visible with the mercury 4358 Å line; then the objective and condenser were displaced to the focus for the desired wave length in the ultra-violet by means of a micrometer screw with a calibrated drum-head reading to microns. Calibration charts were plotted in terms of the displacements necessary to move the objective or condenser from the 4358 Å focus to any of the ultra-violet wave lengths used. It was not essential to make more than one setting for focus except for the immersion objectives, and then only when interested in detailed structure.

Fig. 1-5.—Fig. 1. Cross section showing the variation in the length of the radiation path through an absorbing layer, D, when a convergent beam is employed. Path is given by  $D/\cos u$  for any angle u.—Fig. 2. Cross section of the optical system of the quartz microscope as used for photography. MS, monochromator slit; M, mirror for employing auxiliary source for focusing; C, condenser; OB, objective; E, eyepiece; CAM, film camera; PF, platinum filter covering half of exposed film.—Fig. 3. Variation in the measured transmission  $I/I_0$ , as a function of the angular aperture of the convergent cone, for various values of kD. The value for parallel radiation is shown at O angular aperture. —Fig. 4. The percentage error in k as a function of kD, when  $I/I_0$  is measured at the various angular apertures indicated for each curve.—Fig. 5. Characteristic optical densities of the photographic film when plotted against log(I), where I is the percentage transmission of the calibrated platinum filters or of the absorbing sample, respectively.

An 85 watt high pressure mercury arc (General Electric Vapor Lamp Company type H3) proved convenient as an auxiliary source of visible radiation, enabling one to focus on a ground glass. This arc was placed at the side so that its radiation could be reflected into the condenser at will by means of the mirror M shown in figure 2. Corning glass filters were used to isolate the 4358 Å line.

A water-cooled capillary mercury arc operating at atmospheric pressure furnished the ultra-violet radiation, which emerged from a quartz window in the side of the brass water cell. Distilled water was used for cooling. The arc had a power consumption of about 200 watts, the current being 4 amperes. A slit in the water-cooling chamber served as an entrance slit for the Hilger medium quartz spectrograph which was used as a monochromator. In order to keep the microscope in permanent alignment, it was found convenient to reverse the usual optical path through the spectrograph. For this purpose, the plate holder at the camera end of the spectrograph was replaced by a sliding attachment which carried the arc and its water-cooling cell. Desired wave-lengths were obtained by moving the arc along the position normally occupied by the spectrum plate. Mercury lines used were: 3650, 3341, 3130, 3022, 2967, 2894, 2804, 2652, 2537, 2483, and 2378 Å.

Methods of measurement.—Three methods of making absorption measurements have been used, all of which require at least a microscope condenser, and two make use of a complete quartz microscope. The first method consisted of focusing a small spot of radiation about .035 mm. in diameter on the object to be investigated and measuring the incident and transmitted flux by means of a Geiger-Mueller photoelectric counter. The counter-tube had a cylindrical cadmium electrode within a quartz envelope filled with hydrogen or helium. The photoelectric threshold of cadmium is around 3500 Å so that visible light does not interfere. Since the irradiated spot was extremely small, the counter-tube was built to correspond, the cathode being only 6 mm. in diameter. The counting circuit was a scale-of-eight type, similar to the one described by Shepherd and Haxby (1936). The principal disadvantage of the Geiger-Mueller arrangement, apart from experimental difficulties, was the time factor, for each single observation required counting for a minute or more, while a complete absorption determination at a single wave length often demanded up to ten minutes. On the other hand, radiation intensities could be kept low. The final data reported in this paper were not obtained with the counter-method, but preliminary results with the counter were corroborated by the results given here.

In the photographic method of measuring absorption, a roll-film camera was employed on the microscope so that 32 pictures could be taken on each film, thus enabling one to make several exposures at each wave-length investigated, varying either the focus or the exposure time. Placed immediately in front

of the film and covering half of each exposed strip was a calibrated platinum step filter (P.F. in fig. 2). Hence, for every exposure, a graded series of photographic densities for comparison was available. In practice, the densities of these photographic images of the clear field, produced by ultra-violet radiation whose intensity was known in terms of the original incident intensity, were measured with a microphotometer and plotted as ordinates on a graph with the logarithm of the ultra-violet radiation intensities as abscissae. These intensities were given by the percentage transmission of the various platinum filters.

Figure 5 illustrates a typical characteristic curve for the photographic film and shows how the transmission for a pollen wall can be interpolated from it when the density of the wall image is known. In practice, it was customary to photometer the image of the wall first, then to photometer only the two filtered images of the field having densities immediately higher and lower, respectively, and interpolating by means of a straight line. Two or more exposures were usually made for different lengths of time at each wave length and the results averaged.

Investigators familiar with photographic spectrophotometry will realize the great practical advantage in having comparison standards along with every exposure, each exposure being at a single wave length. In this way, one avoids entirely the variations in the characteristic curves of the film which result from their dependence on wave length, emulsion, kind and extent of development.

The merit of the platinum filters or step-weakeners for decreasing the intensity of the direct radiation beam is owing to their practically constant transmission over a wide wave length range. Actually the transmission does increase slightly with decreasing wave length (see Follett, 1936). Calibration measurements for the particular platinum step-weakeners used here are given in table 1.

Table 1. Transmission in percentage of platinum stepweakeners of varying thickness.

Wave-length	1	2	3	4	5
3650 Å	4.54	9.9	14.6	29.9	40.2
3130 Å	4.14	10.0	14.5	30.5	41.5
2804 Å	4.06	10.9	15.8	30.8	41.6
2537 Å	4.23	9.8	15.6	30.8	40.6
2399 Å	6.38	12.3	18.3	34.6	44.0

These step-weakeners have been prepared by cathodic sputtering of platinum on thin slips of crystal quartz about 6 mm.  $\times$  25 mm.  $\times$  0.6 mm. in thickness. Properly protected surfaces will retain their calibration indefinitely.

In using the photoelectric method for absorption measurements, the photographic plate was replaced by a gas-filled caesium-oxide-on-silver photocell. The spectral sensitivity of this photocell has been described by Young and Pierce (1931). The 6 mm.

dry objective was employed in combination with the lowest power condenser (N.A. = 0.35) in order to secure a longer working distance. To increase the radiation intensity at the image, magnification was kept at a minimum with the 5×eyepiece.

The photoelectric current was amplified with a direct current amplifier of the type described by Penick (1935), using the Western Electric tube no. D-96475. The grid-leak resistance was 10<sup>8</sup> ohms. A high sensitivity galvanometer in conjunction with a lamp and scale at 2 meters distance gave ample deflections, the stronger lines such as 2650 Å requiring only 0.01 of the full sensitivity.

The sample being measured was placed on the stage so that it could be rapidly displaced between two fixed stops, one stop permitting a measurement through the sample, the other through a control solution.

Description and preparation of pollen.—Pollen grains of Zea mays are approximately spherical with a mean diameter of 93 microns. The walls are thin and smooth, usually contain one pore surrounded by a thickening in the wall. Under high magnifications, however, the walls exhibit a stippled appearance. The nuclei of mature pollen are three in number, two generative nuclei having a long sperm-like structure (Rhodes, 1934; Weatherwax, 1919) and a vegetative nucleus about  $10~\mu$  in diameter (Weatherwax, 1919).

The moisture content of corn pollen is high, approximating 60 per cent. Of major importance is the reserve food supply which consists primarily of small starch granules. These starch grains are very effective scattering centers for radiation.

Pollen was obtained from freshly opened anthers grown in the greenhouse. For measurements on wall transmission, an individual grain was crushed in distilled water, where the wall was freed from its protoplasmic contents, and then removed to a 50 per cent mixture of glycerine and water on a quartz slide. A coverslip was added, care having been taken to see that a single layer of wall was present. Measurements of  $I_0$  were taken through the glycerinewater layer adjacent to the sample of wall.

Determinations of transmission by the contents of pollen grains were made by crushing out the contents to form a layer 8 microns thick. This layer was free from walls in the region where measurements were taken. Thickness of the layer was controlled by spacers of aluminum foil placed between quartz slides, the latter being held together between brass plates. A vaseline seal prevented evaporation. In this case, too, the control intensity,  $I_0$ , was measured through a 50 per cent glycerine-water layer of the same thickness as the contents.

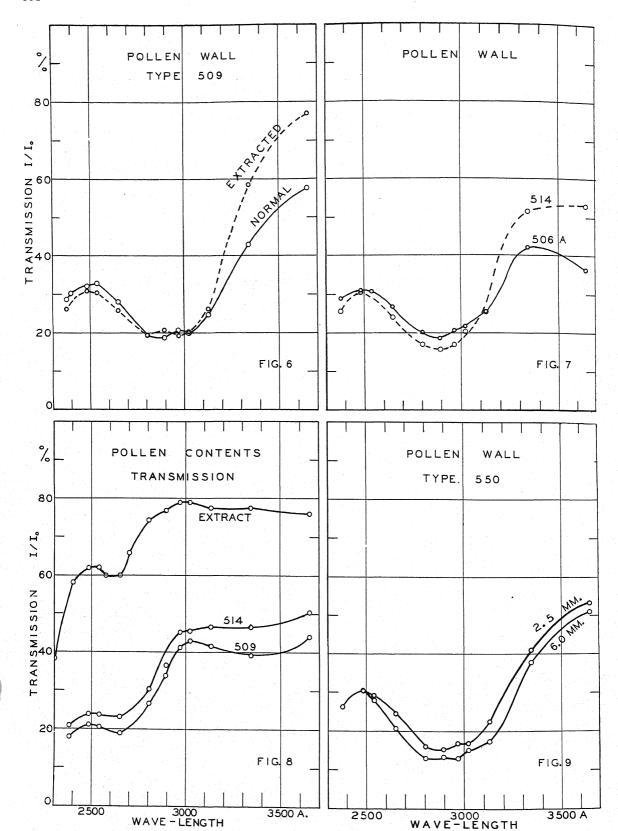
The pollen came from several genetic stocks which had been used extensively in a study of the genetic effects of ultra-violet radiation (Stadler and Sprague, 1936; Stadler and Uber, 1938). Family 509 was a purple variety with the homozygous dominant characters "A B Pl." Family 514 was characterized by the genetic markers "A R j L" and was

of interest because it had given some indication of reacting differently when treated with ultra-violet. Family 550 had the markers "A C r/rs v gl lg j." Family 506A-4 was "Nojoya teosinte," the pollen grains of which were rather variable in size, but always much smaller than corn pollen. The larger grains only have been used. Since the teosinte (Euchlaena mexicana) is a genus related to Zea, it offered the possibility of possessing a different absorption spectrum, either qualitatively or quantitatively. Nothing regarding the chemical composition of these particular varieties of pollen is known.

Experimental results.—Pollen walls.—Transmission curves for a single thickness of wall, with measurements made only at the prominent mercury emission lines, are shown in figures 6 and 7 for various pollen wall samples. In figure 6, family 509 is represented by two curves, one being for normal walls, the other for walls extracted to remove pectin and water-soluble constituents. These two curves do not differ appreciably except at the two longest wave-lengths where observations were made. The curve for the extracted walls is an average of two sets of data obtained from two pollen walls extracted in different ways. Both gave closely similar results. The first method of extraction consisted of treating the walls with a 0.5 per cent aqueous solution of sodium citrate for 30 minutes at 80°C. The second method comprised autoclaving the walls at 120°C. for 30 minutes in 2 per cent HCl, rinsing in distilled water, and then autoclaving again under the same conditions, but with the walls in 2 per cent KOH. Following another rinsing in distilled water, the walls were placed in the usual 50 per cent glycerine-water mixture preceding the transmission measurements. The only apparent result of the extraction was to remove substances possessing absorption bands in the region extending from 3130 Å to longer wave-lengths. This question will be discussed in a later paragraph.

The curve for the normal walls of family 509 represents the average of eight trials, each trial consisting of a complete spectral transmission curve for a single pollen wall. Normal as well as extracted pollen walls from family 509 exhibited a broad region of minimum transmission centering around 2900 Å and extending from 2800 to 3000 Å. This broad minimum was also characteristic of the "Nojoya teosinte" (family 506A) and families 514 and 550, the latter two being represented in the curves of figures 7 and 9, respectively. The teosinte pollen, however, had a minimum transmission at 3650 Å. This would seem to indicate the presence of a water-soluble substance with an absorption band in the vicinity of 3650 Å, possibly a pigment.

All pollen samples measured had similar transmission curves at the shorter wave-lengths, all having a maximum at 2500 Å. Not only the qualitative features of the curves agreed, but also the quantitative data are in substantial agreement. Families 514 and 550 gave somewhat lower minimum values of transmission.



It has already been stated that the transmission values apply to pollen walls immersed in a liquid. For a dry wall in air, there would be an additional reflection loss amounting to around 4 per cent for each air-wall interface. This means that the ultraviolet radiation which could penetrate to the protoplasmic layer in contact with the inner side of the wall of a pollen grain in its natural condition would amount to about 96 per cent of the values given in the accompanying curves of figures 6, 7, and 9.

Pollen contents.—Transmission curves for the heterogeneous contents of corn pollen, measured photoelectrically on 8 micron layers of the crushed grains, but excluding wall material, are reproduced in figure 8. The curve for family 514 is an average of five trials, all on pollen from the same plant. Similarly, the family 509 curve represents the average of six trials, a complete wave-length curve on a single pollen sample constituting a trial. A minimum in the transmission occurs around 2650 Å and a maximum around 2500 Å. It will be noted that the transmission in the long wave-length portion of curve is relatively independent of the wave length. This type of curve has been checked by all three methods of measuring intensities—that is, the photographic, the photoelectric, and the Geiger-Mueller counter method. Also an extract of the pollen in a phosphate buffer solution at pH 6.8 shows the same characteristics, as can be seen from the top curve of figure 8. The minimum at 2650 is somewhat more pronounced here since the transmission is higher and no starch granules are present to scatter radiation.

An apparently significant, though slight, difference in the absolute values of the transmission for the two types of pollen contents has been found. This difference is not a function of wave-length and could be due to variations in starch concentration.

DISCUSSION.—Pollen walls.—That plant cell walls absorb ultra-violet radiation has been known ever since the publication of Köhler's (1904) original photographs with the quartz microscope. It had been suspected years before that such was the case and particularly that the outer walls of plants constituted a protection against the ultra-violet radiation in sunlight. Köhler had used only the wave-length 2750 Å, and Schultz (1910), in an extensive survey of plant tissue, confined his photography to 2800 Å. But these wave-lengths are probably never present in sunlight reaching the earth's surface. It may be significant that the only radiation in sunlight capable of producing genetic alterations in corn pollen (Stadler and Uber, 1938) has now been shown to have minimum transmission through the pollen walls. Investigations by Stadler and Sprague (1936) have already shown that sunlight is probably not responsible for the natural mutation rate in corn.

Schultz's (1910) finding that the epidermis and cuticle of leaves are relatively very opaque to 2800 Å has been recently confirmed by Meyer (1938) who claimed that cutin itself is probably responsible for the ultra-violet absorption. Likewise Frey (1925-1926) ascribed to "cutin" the absorbing rôle for leaf epidermis and stated that the cellulose micelles are transparent to ultra-violet. Neither Meyer nor Frey gave any quantitative data or wave-length information.

Pollen walls are regarded generally as composed of cellulose, cutin, callose, and/or pectic substances. These categories are somewhat indefinite and perhaps not mutually exclusive. According to Möbius (1923) most pollen grains are yellow, the pigment residing in the exine or on the sticky surface in almost all the pollens he examined and only rarely in the interior of the grain. Reeves (1928), in a study of partition-wall formation in the pollen mother cells of Zea mays, found the walls to contain callose but not pectin on the basis of staining reactions. On the other hand, cell walls of meristematic tissue are regarded by Tupper-Carey and Priestley (1923-1924) as made up of a protein in combination with pectin materials, cellulose, and fatty acids. It is evident therefore that the ultraviolet absorption of pollen walls could not be predicted from existing data on their chemical composition. In fact, knowing the absorption curve for pollen walls does not help materially in arriving at wall composition owing to the great dearth of absorption data for the separate chemical constituents. Absorption curves for commercial citrus pectin show a maximum at 2800 Å. This may mean that pectin is not present in the walls of corn pollen, but it is not conclusive. However, such a finding would be in agreement with Reeves (1928). Likewise, the results of extracting pollen walls for pectic substances indicates that pectin is not present. Lignin, too, seems excluded if one compares the pollen curves with those of Herzog and Hillmer (1931) on lignin derivatives, who reported maxima around 2800 Å.

The only other known data on absorption by pollen has been reported by Noethling and Stubbe (1934) for Antirrhinum majus. They used a suspension of pollen grains in 30 per cent ethyl alcohol and obtained a curve very similar to the one given here for the walls only of Zea pollen. Preliminary results on whole Antirrhinum sp. pollen grains by the photomicrographic method do not confirm the curve of Noethling and Stubbe for the pollen suspension. It seems probable that in the suspension method the absorption by the walls predominated, as might be anticipated, since the ellipsoidal Antirrhinum grains swell up to a spherical shape in 30

Fig. 6–9.—Fig. 6. Transmission curves for individual pollen walls, normal and after extraction for pectin and water soluble constituents. 2.5 mm. objective.—Fig. 7. Transmission curves for individual walls of two distinct types of pollen; 514, corn pollen; 506A, "Nojoya teosinte." 2.5 mm. objective.—Fig. 8. Transmission curves for 8 micron layers of pollen contents for types 509 and 514 of corn. The top curve is for an aqueous pollen extract in phosphate buffer solution at pH 8. 6.0 mm. objective.—Fig. 9. Pollen wall transmission curves as measured with 2.5 mm. and 6.0 mm. focal length objectives. See text for comment.

per cent alcohol, and the pollen contents tend to contract into a small volume.

Pollen contents.—The low values for the transmission of ultra-violet by thin layers of contents is owing in part to the scattering of radiation by the starch granules. However, the selective absorption, particularly at wave-lengths shorter than 2967 A, is very marked with a minimum occurring in the neighborhood of 2650 Å. Curves indicating absorption in this region are not peculiar to pollen protoplasm but are characteristic of a number of organisms such as bacteria (Gates, 1930), yeast (Landen and Uber, 1939), sea urchin eggs (Vles and Gex, 1934), sporidia of Ustilago zeae (Landen, 1939), and the cytoplasm of Drosophila eggs and onion root tips (Caspersson and Schultz, 1938, 1939, respectively). Caspersson (1936) envisages a nucleic acid metabolism associated with cytoplasmic processes and ascribes the absorption to nucleotides or nucleoproteins. Absorption by starches and sugars is not sufficiently intense in this region to account for the curves obtained even if they did happen to possess selective absorption at the correct wave-lengths.

Chemical analyses of corn pollen have been made by a number of workers. The bulk of the pollen grain consists of water, starches, and sugars. Other substances found by Anderson and Kulp (1922) and Anderson (1923) were inosite, choline, l-proline, nonakosane (C<sub>29</sub>H<sub>60</sub>), phytosterol, and various phosphatides. Adenine gave them a negative test but has since been identified in corn pollen by Vinson (1927) and Miyake (1922, 1924). In addition Vinson reported arginine, lysine, tyrosine, β-hydroxyglutamic acid, a glutelin fraction, and a crystalline pigment believed to be a flavone or a flavonal. The corn protein, zein, is probably present. Its amino acid composition includes in decreasing order of importance the following: glutamic acid, leucine, proline, phenylalanine, tyrosine, alanine, β-hydroxyglutamic acid, valine, aspartic acid, arginine, serine, histidine, and cystine.

Of the various components named, those known to have selective absorption in the ultra-violet region in question are adenine with a maximum at 2600 Å, zein with a maximum at 2750-2800 Å and corresponding presumably to its tyrosine and phenylalanine content, and the flavone pigment with an absorption band probably around 3300-3600 Å. Adenine is associated with nucleic acid and may be present in that form or as a nucleo-protein. It seems probable that the constituents just named, which are known to be present, account for the major part of the absorption. Since numerous substances have absorption bands in the region in question, and since the composition of pollen is so complex, it is obviously impossible to make a specific analysis on the basis of absorption data alone. This is especially true when the necessary absorption curves are not even available for the known pollen constituents in their pure state. Of the substances which may be present in minute amounts, and thus contribute to the totality of the transmission picture, nothing can be said.

Whole grains.—It may occur to some that measurements might have been made to better purpose by using intact pollen. Apart from technical difficulties due to the shape and size of a grain, it will now be seen from the data obtained for thin layers of contents and for the walls that such measurements would be practically impossible. A central section through a whole grain comprises at least eleven of the 8-micron layers which were employed for measurement. Since each layer transmits only 20–50 per cent of the radiation incident upon it, depending on the wave-length, an entire grain could transmit but a very small fraction of 1 per cent.

In addition to the analytical value of absorption data with reference to chemical composition, it has perhaps a more important place in connection with genetic irradiation studies. In the latter connection, computation of the energy actually reaching the nuclei or chromosomes of the pollen depends on a knowledge of the transmission curve for both the wall and the contents. Calculations of this sort for corn pollen are complicated by the fact that the two generative nuclei occupy eccentric positions in the grain so that no simple statement can be made with respect to the transmission of radiant energy to the nuclei. Detailed consideration of this problem will appear in a forthcoming paper by Dr. L. J. Stadler and the present writer.

### SUMMARY

Theoretical considerations involved in the measurement of absorption spectra with the microscope are discussed.

Photoelectric, Geiger-Mueller counter, and photographic methods of ultra-violet spectrophotometry with the microscope are described, the photographic method employing step-weakeners of platinum on quartz as a basis for density determinations.

Walls of Zea mays pollen grains of varying genetic constitution have similar transmission curves, characteristic features being a broad minimum centering around 2900 Å, a maximum at 2500 Å, and some variation among pollen varieties in the region 3200-3600 Å. A single wall transmits 15-20 per cent at 2900 Å.

Contents of pollen grains are characterized by a minimum in transmission around 2600 Å, a maximum at 2500 Å, and a fairly low but rather constant transmission from 3000 Å to 3600 Å. An 8 micron layer of pollen contents transmits 19–23 per cent at 2650 Å.

Only a limited correlation of corn pollen absorption spectra with chemical constitution is possible at present owing to a lack of adequate chemical analyses and essential absorption data for the individual components present.

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# FACTORS INFLUENCING THE EFFICIENCY OF PHOTOSYNTHESIS <sup>1</sup>

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Warburg and Negelein (1923) reported that Chlorella, when grown under suitable culture conditions, could assimilate carbon dioxide at the rate of 0.25 molecule per absorbed quantum of visible light, a yield close to the supposed theoretical limit. Before this quantum yield becomes an acceptable basis for theoretical discussions of the assimilatory mechanism, three questions require consideration. First, is the value reproducible in other laboratories? Second, can still higher values be obtained? And third, do the measured values truly represent the quantum yield of the assimilatory mechanism?

Although Manning, Stauffer, Duggar and Daniels (1938), using methods different from Warburg and Negelein's, found considerably lower yields, the first question has been answered in the affirmative by Rieke (1939), who obtained yields close to 0.25 by following the technique of Warburg and Negelein in detail. The second and third questions are dealt with in the present paper. Part I shows that the efficiency of photosynthesis is critically dependent on a number of factors, and that favorable combinations of these factors lead to quantum yields as high as 0.33. Part II presents experimental evidence that two assumptions upon which ordinary manometric measurements of photosynthesis depend do not hold for the conditions of the quantum yield measurements, so that the published values should be regarded as characteristic of the method by which they are obtained, rather than of the assimilatory mechanism

Our experimental technique and method of calculation of results are explained in Part III. The general procedure was the same as that of Warburg and Negelein. Liquid cultures of Chlorella were grown to a suitable density, harvested, washed, and subjected to manometric measurements of photosynthesis in sodium light ( $\lambda = 589 \text{ m}\mu$ ) of known energy content. The pressure change due to photo-

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synthesis was computed from observations made during alternating ten-minute periods of light and darkness, and from this pressure change the photosynthesis was computed, exactly in the manner of Warburg and Negelein. As will be shown in Part II, the values obtained in this way are probably not true efficiencies but may be anticipated to be proportional to the true values. In Part I, where we discuss the influence of various factors on efficiency, we mean only the efficiency measured by this particular system of computation.

PART I. CONDITIONS LEADING TO HIGH EFFICIENCY. -Composition of the culture medium.—The addition to the medium of small amounts of certain substances may profoundly affect the efficiency of cells grown in the medium. Traces of organic materials (yeast extract, adenine, uric acid) sometimes increased growth but failed to improve the quantum yield. Traces of inorganic materials (particularly various heavy metals) caused increases in efficiency as well as in growth. Little attention has heretofore been paid to heavy metals in connection with the quantum yield of photosynthesis, probably because the necessary traces have usually been included in the salts and water used to prepare the medium. But failure to obtain high efficiency may be due to the use of salts and water which contain too little of the essential heavy metals. If very pure water is used, it becomes necessary to supplement the ordinary formula for the culture medium. We have found that the addition of an extra amount of ferric sulfate may greatly increase the efficiency of cells grown in a given medium. It has been possible to show that this improvement is not due to the iron itself, but to impurities contained in abundance in this particular iron salt. Indications of the identity of the impurities responsible have been obtained by direct addition of traces of eleven different elements to the culture medium.

Table 1. Comparison of efficiency and growth with two iron concentrations and with water from various sources. Efficiencies in all tables are averages of results from two successive light exposures. Except in table 3, the age given includes three days at reduced intensity.

		Ferri	c sulfate	0.5 ×	10-5 n	nolar	Ferric sulfate $2.5  imes 10^{-5}~\mathrm{molar}$				
Water	Quantum molecule per quan	${ m s~CO_2}$	Absorp- tion per cent	d	ulture ensity m.3/ml	cu		Quantum yield molecules CO per quantum	<sub>2</sub> tion	Culture density mm.3/ml.	Age of culture days
Searsville Lake	.183		95.		1.53		7	.183	98.	1.74	7
Baltimore tap	.221		95.		1.43		7	.261	98.	1.62	7
Laboratory tap	.192		98.		1.76		8	.267	100.	2.34	8
Still residue	.194		75.		0.88		7	.288	92.	1.35	7
Laboratory distilled	.160		85.		0.80		9	.222	98.	1.36	8
Glass distilled	.152		80.		0.84		9	.296	98.	1.36	9
Commercial distilled	.262		70.		0.98		9	.300	97.	1.60	9

We shall take up first the importance of the source of the water and the amount of iron added to the medium. Then evidence will be presented to show that the effect of both the iron and the waters is due to traces of elements other than iron. Following this, experiments with the direct addition of traces of various elements will be described.

The influence of the water and the amount of iron salt used to prepare the culture medium.—Table 1 shows the efficiency of photosynthesis (quantum vield) for cells grown in water from seven different sources. The table is divided into two parts, the lefthand part showing the results when the ferric sulfate concentration was  $0.5 \times 10^{-5}$  molar, the righthand part for  $2.5 \times 10^{-5}$  molar. The major nutrient salts were the same in each case. Details of preparation of the medium are to be found in Part III. Both parts of the table show considerable dependence of the yield on the water used, and in every instance except the Searsville Lake water, the yield was appreciably greater with the higher concentration of iron. However, the improvement obtained with the extra iron tends to be smaller in those cases where the lower concentration of iron gave a fairly high vield. The highest yields with the low iron concentration were in Baltimore tap water and commercial distilled water. The increase in yield with five times as much iron was 18 per cent and 15 per cent, respectively. The glass distilled water medium, which might be expected to be most deficient in traces of heavy metals, gave the lowest yield with the low iron concentration and showed an increase of 95 per cent with the fivefold iron concentration. The failure of the yields in Searsville Lake and laboratory distilled water to respond so markedly to the higher iron concentration may have been due to the presence of toxic substances in these waters.

The growth as well as the efficiency depends on the water and the amount of iron added to the medium. Consistent correlation between growth (culture density) and efficiency is lacking, although the higher iron concentration gave greater growth as well as higher efficiency in every case but one.

For comparison with Rieke's yields, and with Warburg and Negelein's, we should look first at our results with the lower concentration of iron, because they specified the same iron concentration for their media (1  $\times$  10<sup>-5</sup> molar ferrous sulfate). In other respects their media were comparable with ours, though not identical. They used twice the concentration of the major nutrient salts in our medium. Since we found no improvement in efficiency with the double concentration of nutrient salts, we regard the iron and the water as the decisive factors. With the low iron concentration only two of our waters gave yields comparable with theirs-Baltimore tap water and commercial distilled water. With the fivefold iron concentration, on the other hand, most of the waters gave yields as good as theirs or better. From our results with the low iron concentration, we must conclude that they were fortunate in their choice of waters. Warburg and Negelein used Berlin tap water, and Rieke used water distilled from a tin still.

Some of the waters listed in table 1 call for explanation. The laboratory distilled water was made by distillation of the laboratory tap water in a tinned still. To make the glass distilled water, the laboratory distilled water was redistilled from an all-Pyrex still. The residue from this still after a number of distillations was the water referred to as still residue. The Baltimore tap water was included because we first thought Rieke had used this in the preparation of his medium. The Searsville Lake water was locally impounded surface water.

In table 1, as well as in subsequent tables, there is a column showing the estimated percentage of the incident light which was absorbed by each cell suspension. This percentage of the measured light entering the vessel was used in computing the efficiencies. The visual method which was used for estimating the absorption, and the resulting uncertainty in the efficiency are discussed in Part III. In the few cases of absorption below 80 per cent the error may be as much as 10 per cent, but for absorption between 95 and 100 per cent, as in most of the measurements, only a negligible uncertainty is introduced.

The elimination of iron as a factor responsible for high efficiency.—It might be supposed that the generally lower yields with the low iron concentration were due to iron deficiency and that in those cases where the yield compares favorably with that in the fivefold iron concentration, the water had supplied the necessary extra iron. But one would hardly expect this to be the case for the commercial distilled water, and figures furnished by the Baltimore City Water Department show that the iron present in Baltimore tap water is only about one twentieth of our low iron concentration. Warburg and Negelein's analysis of their Berlin tap water showed that it contributed only one tenth of the total iron in their culture medium. The dependence of the efficiency on the source of the water must therefore be chiefly attributed to the presence of constituents other than iron. This suggests that the superiority of the fivefold iron concentration is due to traces of impurities added with the iron, rather than to the additional iron itself.

Support for this conclusion is derived from observations of the dependence of growth and efficiency on the age of the culture. The curves in figure 1 show growth and efficiency plotted against age of culture in days, for the low iron concentration (broken curves) and for the fivefold iron concentration (solid curves). In both cases the media were prepared with glass distilled water. If the iron concentration is high, the quantum yield remains essentially constant at a high value throughout the 12 days of the experiment. Growth, though starting slowly, shows an early increase and continues at a uniformly high rate for the same period of time. In the lower iron concentration the young cultures show

an efficiency nearly as great as with the fivefold iron, and a superior initial growth rate. But both the rate of growth and the efficiency drop rapidly after the first few days, as if some essential constituent in the culture medium were becoming exhausted. It seems unlikely that this decline both in growth and efficiency could be due to lack of sufficient iron, because the same medium prepared in a suitable water, which

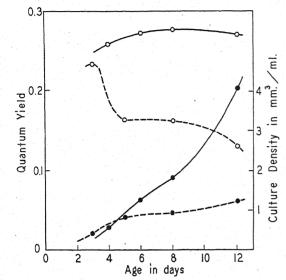


Fig. 1. The dependence of growth (solid circles) and efficiency (open circles) on age of culture. The broken curves are for the low iron concentration (.5  $\times$  10<sup>-5</sup> molar ferric sulfate), and the unbroken curves are for the five-fold iron concentration (2.5  $\times$  10<sup>-5</sup> molar ferric sulfate). Cultures were grown at 900 lux, with no reduction in light intensity. For the measurements at three and four days, several cultures were pooled, in order to obtain sufficient quantities of cells.

as we have seen need not contribute an appreciable amount of iron, will maintain excellent growth and high efficiency to an advanced age. The persistent high growth rate and quantum yield with the five-fold iron concentration must therefore be due to elements other than iron which are carried as impurities in the ferric sulfate. The deficiency which manifests itself early in the development of the culture with the low concentration of iron can be largely made up by the addition of five times as much ferric sulfate, because of the impurities contained in the ferric sulfate.

If the beneficial effect of larger amounts of iron is due to impurities, then for a given amount of iron, the efficiency should depend upon the purity of the iron source. Table 2a shows the efficiency for four cultures, grown in glass distilled water media, each of which received the same amount of iron, but from four different sources. In general, both growth and efficiency were better, the lower the purity of the iron. The ferrous tartrate, furnished by the plant nutrition laboratory of the University of California at Berkeley, had been specially purified to free it from traces of heavy metals. This gave the lowest efficiency and poorest growth. The fact that the reagent ferric sulfate, presumably of higher purity than the c.p. grade, gave the highest growth and efficiency may be an indication that toxic as well as beneficial impurities are involved; but it must also be remembered that the standards of purity of chemical reagents may bear no consistent relation to the minute traces of elements important in plant nutrition (Arnon and Stout, 1939, p. 145).

The direct addition of microelements.—The low efficiency characteristic of the low iron concentration in glass distilled water can be increased not only by the addition of more iron or the use of a more

'Table 2. a, Dependence of efficiency upon source of iron. b, c, Effect on efficiency of addition of microelements to medium with low and with high iron concentration. All media were made up in glass distilled water, Quantities of supplementary solutions are per liter of medium.

Iron and microelements in the culture medium	Quantum yield, molecules CO <sub>2</sub> per quantum		Culture density mm. <sup>3</sup> /ml.	Age of culture days
. Iron 1 × 10 <sup>-5</sup> molar, as				
ferrous tartrate	.092	75.	0.61	13
ferric sulfate, c.p	.144	80.	0.66	8
ferrous sulfate, reagent	.163	80.	0.62	9
ferric sulfate, reagent	.186	92.	0.88	9
. Iron 1 × 10 <sup>-5</sup> molar, as ferric sulfate, c.p.				
with 1 ml. B6	.186	85.	0.68	9
with 1 ml. A5	.264	98.	1.36	9
with 1 ml. A5, 1 ml. B6 ferrous tartrate	.296	92.	1.07	8
with 1 ml. A5, 1 ml. B6	.223	100.	1.66	10
Iron $5 \times 10^{-5}$ molar, as				
ferric sulfate, c.p	.280	95.	1.05	8
with 0.2 ml. A5, 1 ml. B6	.327	100.	1.49	8
with 1.0 ml. A5, 1 ml. B6	.295	99.	1.44	9
with 5.0 ml. A5, 1 ml. B6	.299	100.	2.36	20

favorable water, but also by the direct addition of certain elements to the medium. The elements chosen were those found by Hoagland and his collaborators to be important in the culture of higher plants and designated by them as microelements (cf. Hoagland, 1937; Arnon, 1938). They very kindly furnished us with two stock solutions containing elements grouped according to their importance. Supplementary solution A5 includes those elements now known to be essential for normal development of certain higher plants (boron, manganese, zinc, copper and molybdenum), while B6 contains elements not yet known to be essential, but known to be definitely beneficial when used as a group (titanium, vanadium, tungsten, chromium, cobalt and nickel). The concentrations of the several elements in the supplementary solutions are the same as specified by Arnon (1938) for solutions A4 and B7. The change of designation to A5 and B6 is due to the recent transfer of molybdenum from the B to the A group. To each liter of medium, Arnon recommends the addition of 1 ml. of each of the supplementary solutions, giving concentrations in the medium ranging from 0.5 parts per million for boron down to 0.01 ppm. for the elements in the B6 solution.

Table 2b shows the effect of adding these quantities of A5 and B6 solution to the medium prepared in glass distilled water with low iron concentration. A5 improves the efficiency 85 per cent, and B6, 29 per cent. Used in combination they increase the quantum yield more than 100 per cent, so that it becomes comparable with the highest yields shown in table 1 for the fivefold iron concentration. It seems not unlikely that the A5 and B6 solutions include some of the same elements responsible for the improvement in yield brought about by the use of favorable waters, or of extra amounts of iron.

Table 2b also shows that the very low yield with iron as ferrous tartrate can be increased 100 per cent by addition of A5 and B6 solutions. The level attained is not so high as when the iron is added as ferric sulfate, probably because the latter contains elements lacking both in the tartrate and in the A5

and B6 solutions, or because the impurities in the ferric sulfate together with the A5 and B6 solutions give a more favorable combination of microelements than the A5 and B6 solutions alone. The possibility also remains that the iron as tartrate is not equal to sulfate in physiological value.

Even the yield obtained by using the fivefold iron concentration can be increased by the addition of microelements. Table 2c shows the effect of three different concentrations of the A5 solution added to the medium in glass distilled water with high iron concentration. The amounts of A5 added were 0.2, 1, and 5 ml. per liter of medium. In each case 1 ml. of B6 was used per liter, as before. The lowest concentration of A5 gave the best results, with an increase of 18 per cent in growth and 17 per cent in efficiency. The two higher concentrations gave only small increases in efficiency over the control (5 and 7 per cent) and inhibited growth. The inhibition of growth, scarcely evident with the middle concentration, was so extreme with the highest concentration that the culture grew hardly at all for the first 10 days. After this it began to grow faster and had reached a good density in 20 days. It is interesting to note that the quantum yield was high in spite of the delayed growth. The inhibition of growth with high concentrations of A5 solution is reminiscent of the slower initial growth characteristic of the cultures with high iron concentration, shown in figure 1. Although this might have been caused by an excess of iron, the growth inhibition due to the A5 solution suggests that it was caused by an excess of some microelement carried by the ferric sulfate as an impurity.

The critical dependence of the growth, and, to a lesser extent, of the photosynthetic efficiency on the amount of A5 solution added to the medium is an indication that certain of the constituents may be somewhat toxic even in the lowest concentration used. Independent variation of the concentration of the individual microelements may be expected to lead to still higher quantum yields.

Table 3. Dependence of efficiency upon light intensity during culturing and during measurement. All cultures were grown in medium with ferric sulfate 2.5 × 10-5 molar in glass distilled water. 0.2 ml. A5 and 1.0 ml. B6 micronutrient solutions were added to all but the first culture, which received equivalent quantities of only manganese, copper, and zinc.

	Quantum yield	l at intensity of			
Light intensity during culturing Source Lux	350 ergs/cm. <sup>2</sup> /sec. molecules CO <sub>2</sub> per quantum	$1500$ ergs/cm. $^2$ /sec. molecules $CO_2$ per quantum	Absorption per cent	Culture density mm. <sup>3</sup> /ml.	Age of culture days
4 60-watt bulbs at 10 cm. 6700	.102	.096	100.	3.24	6
1 100-watt bulb at 10 cm. 3100	.216	.175	98.	2.16	5
1 100-watt bulb at 20 cm. 920	.275	.212	100.	1.80	8
1 100-watt bulb at 30 cm. 440	.263	.181	95.	1.01	9
920 lux 7 days, 440 lux I day	.333	.232	95.	1.54	8
920 lux 7 days, 440 lux 3 days	.336	.195	98.	1.86	10
920 lux 7 days, 440 lux 5 days	.324	.206	98.	2.45	12

CONDITIONS DURING GROWTH OF CULTURES .--Light intensity .- Table 3 shows that the culturing of cells at high light intensities (6700 lux) is unfavorable for development of high photosynthetic efficiency, although growth is much faster. The existence of an optimum in the neighborhood of 900 lux is indicated. Reduction in light intensity a few days before harvesting, a procedure recommended by Warburg and Negelein, results in a decided increase in efficiency. If the initial period of growth takes place at 900 lux, the table shows that the maximum effect of a 50 per cent reduction occurs after about three days. The preceding period of growth at the higher intensity is important, because if the entire growth takes place at the lower intensity, the efficiency is no better than after growth at 900 lux without reduction. Other combinations of intensities failed to give promise of higher efficiencies. The practice we generally followed, growing the cultures to a moderate density at 20 cm. from a 100-watt bulb and then moving them to 30 cm. for three more days, was the same as specified by Rieke.

Age of the culture and the interdependence of various factors.—As a culture grows older, there is a gradual automatic reduction in the average light intensity within it, due to absorption by the cells. Some tendency of the efficiency to increase with age may therefore be anticipated independently of reduction in the incident intensity (cf. fig. 1). The reverse tendency for the efficiency to decline as essential constituents of the medium become exhausted has already been discussed. The age at which this decline appears may be expected to depend on the light intensity used for growing the cultures, because the faster growth at higher light intensities will result in earlier exhaustion of the least abundant elements. Such an effect has been observed in some of the experiments where cultures were grown at very high light intensities.

Wave length.—Without adequate control of medium, light intensity, and age of the cultures, the dependence of efficiency upon the wave length distribution of the light used for culturing the cells cannot be satisfactorily investigated. Before the interaction of these factors was appreciated, a number of cultures were grown in the blue and yellow mercury lines ( $\lambda = 436$  and 478 m $\mu$ ) and in various regions of the neon spectrum. The results indicated considerable dependence of the quantum yield on the wave

length of light in which the cells were grown. We believe it possible that with proper attention to other factors, control of the wave length may lead to further improvement in the quantum yield.

Temperature.—The temperature at which the cells are grown appears to play relatively little part in the efficiency of photosynthesis. Cultures grown at 10° showed 7 per cent lower efficiency than cultures of corresponding density grown at 20°. No measurements were made with cultures grown at temperatures appreciably higher than 20°. Most of the cultures were grown between 17 and 22°. In any one series the temperature was maintained constant within about 2°.

Conclusions from experiments on culture conditions.—In seeking to identify the culture conditions which determine the efficiency of the photosynthetic apparatus, we have accumulated evidence that optimal adjustment of any one factor depends somewhat on other factors. We have tried as far as possible to combine the factors in such a way as to obtain the highest possible efficiency, but it has been necessary to make arbitrary choices in regard to certain factors, in order to study the influence of others. Further attention to the interdependence of the various factors may be expected to lead to higher values for the photosynthetic efficiency.

CONDITIONS OF MEASUREMENT OF PHOTOSYNTHEsis.—Temperature.—Turning now from the medium and culture conditions to the conditions during measurement of photosynthesis, we have again found a fairly critical dependence of efficiency on a number of factors. The highest efficiencies have been obtained at a temperature of about 10°. Table 4 shows how the yield falls off at temperatures both above and below 10°. Warburg and Negelein, and Rieke, carried out their measurements at 10°, while Manning et al. worked at  $25\,^{\circ}$  for the most part, and made a few experiments at  $1\,^{\circ}$ . From the figures in table 4 one might expect the efficiency at these temperatures to be less than half that at 10°. Wassink, Vermeulen, Reman, and Katz (1938) report quantum yields for Chlorella running from 0.11 to 0.20 at temperatures from 10° to 29°. It appears from their curves that the highest yields were at the higher temperatures, instead of at 10° as in our experiments. However, their purpose was to compare fluorescence and photosynthesis, and the quantum yields were only incidental. Their conditions of measurement were not

Table 4. Dependence of efficiency on temperature during measurement. All cultures were grown in medium with ferric sulfate 2.5 × 10<sup>-5</sup> molar in laboratory distilled water. The lower yield at 10° for A than for C and D is due to the higher intensity used for measurement. Except for culture D, successive measurements were made with increasing temperature.

	Light intensity		uantum y	yield in	molecu	!	Absorp-		Age of		
~ -	0°	5°	8°	10°	12°	15°	20°	tion density per cent mm.3/ml.	culture days		
$\mathbf{A}$	760	.105	.124		.192				98.	1.68	11
В	760						.133	.088	98.	1.71	11
$^{\mathrm{C}}$	380			.210	.253	.226			98.	1.46	10
$\mathbf{D}$	380				.223	.226			98.	1.51	10

comparable with ours in several important respects. Particularly their light intensity was relatively high, so that it was sometimes less a limiting factor than the temperature. This may explain why they obtained lower yields at 10° than at the higher temperatures, but it does not explain why they were able to get such high efficiencies at the higher temperatures. We should bear in mind the possibility that our own apparent maximum at 10° may be in part connected with our technique of measurement.

Light intensity.—In measuring the efficiency of photosynthesis, the light intensity should be so low that it is the only limiting factor. Proportionality of rate of photosynthesis to light intensity is ordinarily used as a test of fulfillment of this condition. We have made most of our measurements at an intensity of about 350 ergs/cm.<sup>2</sup>/sec. (roughly 300 lux). Intensities lower than this did not give appreciably higher yields. In this intensity range photosynthesis is a nearly linear function of intensity within wide limits. The rate for cells grown in bright light is practically linear over a range of 16 fold, but the efficiency is low. The most efficient cells, which are grown at low intensity, show linearity over a range of two- to four-fold. Greater dependence on intensity would be expected with thin suspensions, where all cells are exposed to the full intensity of the incident beam. In the thick suspensions used for efficiency measurements, most of the cells receive light of much lower intensity than the incident beam. Therefore if the incident intensity is raised to the level where other factors become limiting, the deviation from linearity will be less marked than with a thin suspension. Evidence of deviations from linearity may also be obscured by the use of ten-minute periods of light and darkness. The effect of these short periods is discussed in Part II.

Intermittency of the light.—The sodium lamp used for measuring photosynthesis was ordinarily operated on alternating current and showed a distinct intermittency (flicker). This might be expected to influence the photosynthetic yield at high intensities but should not at low intensities if the yield was, as we supposed, independent of the Blackman reaction. We made some experiments with the lamp operated on direct current, without flicker, and found the yields at 10° were the same as with alternating current. Therefore at this temperature our results are fully comparable with other measurements made in continuous light, in spite of the intermittency of the sodium lamp. But at 0° the alternating current sometimes gave a few per cent higher efficiency, suggesting that at this temperature the Blackman reaction may enter as a limiting factor even at very low light intensity.

Carbon dioxide.—Emerson and Green (1938) showed that the rate of photosynthesis at light saturation was strictly independent of carbon dioxide concentrations from 5 per cent down to 0.01 per cent. On general grounds one would expect less rather than more dependence on carbon dioxide at the very low light intensities used for measuring effi-

ciency. Contrary to expectation, we found that the efficiency was quite dependent on carbon dioxide concentration. It was 25 per cent lower at 0.5 per cent than at 5 per cent. Measurements at 12 per cent seemed to be a little lower than at 5 per cent, suggesting the existence of an optimum in the neighborhood of 5 per cent. We made tests to show to what extent our measurements were comparable with those of Emerson and Green and found that the dependence of efficiency on carbon dioxide concentration was a result of certain of the conditions chosen for measurement of efficiency. Emerson and Green made their measurements at 25° with thin cell suspensions and continuous illumination at high light intensity. Our efficiency measurements were made at 10° with thick suspensions and 10-minute exposures to light of very low intensity. We found that with the customary 10-minute light and dark periods, higher light intensities diminished the dependence of the vield on carbon dioxide, in both thick and thin suspensions. The highest intensity used was 5300 ergs/cm.<sup>2</sup>/sec., 15 times the usual intensity for efficiency measurements, but still far below saturation. With this light intensity, the substitution of long light exposures for the 10-minute periods gave the expected independence of carbon dioxide concentration. With both thick and thin suspensions, the rate of photosynthesis was constant from 0.5 per cent down to 0.07 per cent carbon dioxide, a limiting value in good agreement with unpublished results of Emerson and Green at 10°. This agreement, together with the uniformity of our results regardless of suspension density, shows that the dependence of efficiency on carbon dioxide concentration is not due to inadequate mixing of the suspension. The tests show that it is attributable partly to the low light intensity, and partly to the 10-minute light periods used for measuring efficiency.

Conclusions from Part I.—The second question raised in the introduction, whether quantum yields higher than 0.25 are obtainable, has been answered in the affirmative. By identifying and controlling some of the variables upon which the efficiency depends, it has been possible to obtain yields of 0.33, an increase of 25 per cent. There is a distinct probability that still higher yields could be obtained by further adjustment of conditions. The highest values listed are therefore not to be regarded as the maximum possible yield.

The observed dependence of the efficiency on the conditions of measurement has been somewhat unexpected in several instances, particularly the dependence on temperature and carbon dioxide. Mention has been made of the possibility that the peculiar dependence on some of these factors may result from the technique of measurement. The importance of the technique is discussed in the next section.

PART II. SIGNIFICANCE OF THE QUANTUM YIELDS DERIVED FROM MANOMETRIC MEASUREMENTS.—Theoretical considerations.—Because the quantum yield of 0.25, or four quanta per molecule of carbon diooxide reduced, has been regarded as close to the

theoretical maximum, values higher than this acquire a special interest. Warburg and Negelein believed, as a result of measurements of the ratio of exchange of carbon dioxide and oxygen, that carbon dioxide and water were assimilated directly to carbohydrate, without the accumulation of less reduced intermediate products. The conversion of carbon dioxide and water to carbohydrate, being an endothermic process, requires an amount of energy equal to the heat of combustion of the reverse process. In addition, a margin of energy must be available for thermal steps in the synthesis. It is generally agreed that if all the energy is to be supplied as red light (the long wave length limit of green plant photosynthesis) a minimum of four light quanta must be absorbed per molecule of carbon dioxide in order to meet the energy requirements. Although the energy per quantum is greater for wave lengths shorter than red, Warburg and Negelein found the same efficiency in the yellow mercury line ( $\lambda = 578 \text{ m}\mu$ ) as in red. This is a strong indication of the quantum nature of the photochemical part of the assimilatory process. It is not believed that the energy of a single quantum could be divided between two elementary processes, so if four quanta are the minimum number in red, then less than four cannot be expected to suffice in shorter wave lengths (cf. Warburg and Negelein, 1923, p. 210-212). The close agreement between their measured quantum yields and the theoretical maximum of 0.25 has been regarded as supporting the theory of direct synthesis of carbohydrate from carbon dioxide and water. Various mechanisms have been proposed, in which carbohydrate is synthesized from carbonic acid. Yields appreciably higher than 0.25 would be incompatible with such mechanisms, and, partly for this reason, higher yields have not been anticipated. Our measurements, made in sodium light ( $\lambda = 589 \text{ m}\mu$ ), lead to values definitely higher than the theoretical maximum for complete carbohydrate synthesis from carbonic acid. A yield of 0.33 indicates the absorption of only three quanta per molecule of carbon dioxide. Since values higher than the theoretical maximum are obtainable, we must recognize two possibilities. Either the method of measurement leads to incorrect values, or the chemical mechanism from which the theoretical maximum was deduced is incorrect. First we shall present evidence concerning the reliability of the measurements, and then consider possible modifications of the chemical mechanism which would explain the occurrence of yields higher than

The reliability of the manometric measurements.—The pressure changes caused by photosynthesis cannot be measured directly but are deduced from pressure changes measured in both light and darkness, since correction must be made for the respiration that takes place during the light. It is ordinarily assumed that the rate of respiration during the light exposure is equal to the rate measured in the dark before or after the light exposure. However, it is believed that either the light itself or the process of

photosynthesis may accelerate respiration (cf. Gessner, 1938). In this case the photosynthesis computed by correcting the pressure change during a light exposure with the pressure change during an equal period of darkness will be lower than the photosynthesis actually taking place during the light exposure. Such an error may become serious in measurements of efficiency made over very long periods of light and darkness (Manning, Stauffer, Duggar, and Daniels, 1938).

When measurements are made with low incident light and dense suspensions of cells, the total photosynthesis in the suspension may be less than the total respiration, so that photosynthesis is measured as a decrease in respiration. A small percentage change in the respiration during the light will then cause a large percentage error in the estimated photosynthesis. The assumption that the rate of respiration during a light exposure is equal to the observed rate before or after the light exposure becomes of particular importance. Warburg and Negelein sought to minimize the possibility of error from this source by using short periods of light and darkness, so that the respiration would soon reach a steady rate from which it would not deviate greatly in either light or darkness. They found that periods as short as ten minutes gave readily measurable pressure changes and left sufficient time for readjustment in rate of pressure change following changes from light to dark and vice versa. Nevertheless, they believed that respiration during the light exposures was probably somewhat higher than the measured rate during the dark periods.

We thought it possible that still shorter periods of light and darkness might lead to a closer estimate of the rate of respiration prevailing during the light periods, so we made certain improvements in the apparatus to permit the making of readings at shorter intervals. A cathetometer reading in hundredths of a millimeter instead of tenths made the pressure changes over intervals of a minute or less accurately measurable; and the mixing of the cell suspension was so improved that equilibrium in rate of gas exchange between suspension and gas phase was reestablished within one or two minutes after the light was turned on or off. The readings were made without interrupting the shaking of the manometer and vessels, at intervals of one minute, or sometimes 30 seconds.

The use of shorter periods of light and darkness to reduce errors in the respiration measurement did not prove satisfactory. With our improved technique we found that after a change from light to dark, or vice versa, the rate of pressure change was subject to large deviations before coming to the new steady value. An example of the observed deviations is shown in figure 2, where the rate of pressure change is plotted against the time. To make the deviations clear, the measurements selected for figure 2 were made at a light intensity four times that generally used for efficiency measurements. This gave positive photosynthesis, above the compensation point. At

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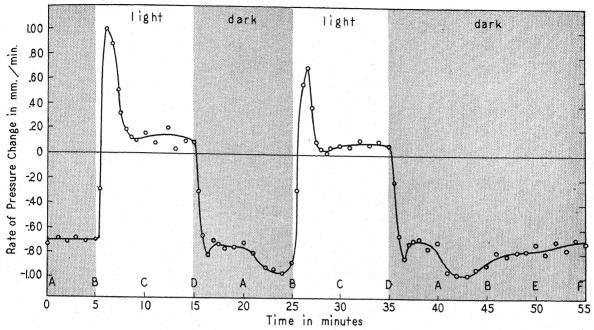


Fig. 2. Deviations from steady rate of pressure change in successive periods of light and darkness. The shaded areas represent the dark periods. Light intensity 1400 ergs/cm.2/sec. The letters A, B, C, etc., designate the times of pressure reading referred to in the text, from which the pressure change due to photosynthesis was computed in various ways.

lower intensity the deviations are much less marked; but we shall show later (table 5) that they still play a significant part in determining the pressure changes from which photosynthesis is computed.

When the light is turned on, a sharp increase of pressure occurs at once and lasts from two to five minutes. Under some circumstances the maximum rate attained may be two or three times the steady rate in the light (the respiration correction being included in each case). It may be noted that, since the mixing in the vessel is not infinitely rapid, the variations in rate of gas exchange at the cells must be even more abrupt than the variations in pressure change recorded by the manometer. When the light is turned off, the rate of pressure change returns approximately to its former (negative) value for a few minutes but then shows an increase. The maximum is reached in about seven minutes and may be 20 per cent above the steady rate, which is regained after 10 to 20 minutes.

These deviations are not due to heat effects, since they disappear when the cells are killed by boiling. Both deviations are affected, though sometimes differently, by the following factors: the conditions of growth of the cells used, the density of the suspension, the light intensity, the partial pressure of carbon dioxide, and the length of time the cells have been in the dark previous to the light exposure. Their dependence on these factors is a strong indication of the physiological origin of the deviations. Values for the efficiency calculated from the observations in figure 2 vary from 0.16 up to the extraordinarily high value of 0.39, depending on whether

photosynthesis is computed from the steady rates, not including any deviations, or from the rates represented by the maximum deviations in both light and darkness. It is not to be supposed, however, that the deviations from steady rate shown in figure 2 are due entirely to irregularities in the rate of photosynthesis or respiration. In part at least, they must be due to changes in the ratio of exchange of oxygen and carbon dioxide.<sup>2</sup> This implies that for the short periods of darkness and illumination used for efficiency measurements the assumptions on which photosynthesis is computed from pressure changes become incorrect.

The computation of gas exchange from pressure measurements with a medium such as ours, in which both oxygen and carbon dioxide are freely exchanged, depends upon a knowledge of the ratio of exchange of these gases. In experiments on photosynthesis, it has generally been assumed that the ratio CO<sub>2</sub>/O<sub>2</sub>, designated by γ, is close to unity. Warburg and Negelein checked this assumption carefully for Chlorella and obtained for the ratio the value -0.9, which they used in calculating their vessel constants. The sign of  $\gamma$  is negative because gas evolution is regarded as positive and gas consumption negative. In order to obtain a sufficient exchange of oxygen and carbon dioxide to permit accurate gas analysis, these measurements were made over periods of one and a half hours or longer, and at high light intensities. The ratio obtained under

<sup>2</sup> Recent experiments have shown that the sudden increase in pressure following the turning on of the light, represented by the peaks between B and C in figure 2, is due principally to evolution of carbon dioxide.

these conditions may not be applicable to the conditions under which high efficiencies have been obtained. The deviations described above indicate that it is not and that the assumption of constant  $\gamma$  is not valid for low light intensity and ten-minute periods of illumination.

The literature already contains a number of observations which indicate that y may vary over a wide range. For example, Gaffron (1939) reports that in media to which sugar has been added algae may under some circumstances accumulate intermediate products of respiration which are probably responsible for deviations in y accompanying the onset of photosynthesis. Recent observations by Blinks and Skow (1938) on the changes in electrical potential of plant cells following changes from light to darkness and vice versa show irregularities similar in some respects to the deviations in pressure change illustrated in figure 2. They have presented evidence that the electrical changes are related to photosynthesis, and among various possible interpretations, they have advanced the suggestion that carbon dioxide may be evolved during the first moments of illumination. They have definite evidence that a sudden burst of oxygen evolution follows immediately upon turning on the light. It must be borne in mind, of course, that such peculiarities in gas exchange may have only an indirect connection with photosynthesis.

Both the uncertainty as to the rate of respiration during the light exposure and the possibility that y may vary over short periods of time furnish grounds for questioning the reliability of efficiency measurements based on manometric determinations of photosynthesis. These two sources of error are in a sense interdependent, since shortening the light and dark periods in order to improve the respiration correction adds to the risk of errors from deviations in  $\gamma$ . The ten-minute periods used by Warburg and Negelein may be regarded as a compromise between these two difficulties. Whether or not it is a good compromise cannot be decided without further knowledge of the behavior of  $\gamma$  under the conditions of their experiments. Our apparently arbitrary decision to follow their schedule of ten-minute periods of light and darkness was reached as a result of observations such as those shown in figure 2.

With a given schedule of timing, the pressure change due to photosynthesis may be computed in several different ways, all of which should lead to the same value were it not for deviations such as those shown in figure 2. It is interesting to note that the method of computation used by Warburg and Negelein was such as to take maximum advantage of the deviations from steady rate. They read the pressure at the times designated A and B in figure 2. Respiration was measured as the change in pressure over the five-minute period from A to B. The fiveminute period from D to A was to permit the carbon dioxide and oxygen exchanged inside the cells during the light exposure to reach equilibrium with the suspending fluid and the gas space. The average of

two successive five-minute periods (A to B) was multiplied by three to give the pressure change due to respiration during the intervening 15-minute period from B to A, which included ten minutes of illumination from B to D. The difference between the observed pressure change for the 15 minutes from B to A and the computed pressure change due to 15 minutes' respiration was assumed to be the pressure change due to photosynthesis during the ten-minute light exposure. This system had the advantage of requiring only two readings, a point of possible importance when the shaking was to be interrupted for each reading.

Photosynthesis can also be computed by making measurements at C and D, as well as at A and B. The rate of pressure change in the light, measured from C to D, can then be corrected with the respiration rate from A to B. Rieke compared the efficiency obtained in this way with the efficiency obtained by Warburg and Negelein's method and found that the latter gave higher yields, a point which may have played a part in their choice of readings. We can confirm Rieke's finding and can show why Warburg and Negelein's periods lead to higher yields. Both the sudden burst of pressure immediately after the light is turned on at B and the low rate of pressure change between D and A tend to raise the computed photosynthesis. The respiration measurement from A to B is the same in both cases and usually includes the maximum deviation of the dark pressure change from the steady rate. The effect of this deviation can be avoided by waiting to make the respiration reading until after the rate has returned to a nearly constant value. For example, the period E to F might be used. But the rate continues to decline slowly in the dark, so that the selection of a period in which to make measurements is still arbitrary; and the longer the time between the light period and the respiration measurement, the more likely it is that the measured rate does not represent the rate of respiration during the light.

Another method of computing the pressure change due to photosynthesis, which should give the same result if there were no deviations, is to measure the average pressure change for five minutes of light from two successive C-D periods, multiply by three, and compare with the pressure change for the intervening 15-minute period D to C, which includes 10 minutes of darkness. In general this leads to a lower value for the efficiency than the other two methods; for the burst of pressure in the light, included in the period D to C, now has the effect of lowering the

respiration reading.

A comparison of the values for two light intensities resulting from four different methods of computation is given in table 5. The differences between these figures bring out the importance of the deviations even at the low light intensities used for measuring maximum efficiency. Until further information is available, it appears to us that the selection of any particular method of computation must be largely arbitrary. This conclusion inevitably tends to lessen

Table 5. Dependence of computed quantum yield on method of computation for two intensities: 350 ergs/cm.2/sec., ordinarily used for efficiency measurements; and 1400 ergs/cm.2/sec., used for figure 2. Letters designating method of computation correspond to those in figure 2.

Met	hod of computation	Computed qua	Computed quantum yield at intensity of					
Interval used rate in lig			1400 c. ergs/cm. <sup>2</sup> /sec.					
B-A	A-B	.328	.209					
C-D	A-B	.306	.184					
C-D	D-C	.292	.158					
C-D	$\mathbf{E}\mathbf{-F}$	.206	.151					

the significance which has been attributed to the grouping of Warburg and Negelein's quantum yields for red and yellow light around the theoretical maximum of 0.25.

Moreover, in this connection it must be pointed out that their system of measuring light intensities involved use of the bolometer in a vertical position, whereas it was calibrated in a horizontal position. Our bolometer, similar in construction to theirs, shows about 12 per cent greater sensitivity in the vertical than in the horizontal position. If their instrument had the same dependence of sensitivity upon position, it would mean that their measurements of light intensity were about 12 per cent too high and their quantum yields 12 per cent low. The values then would no longer be so closely grouped around 0.25.

The third question raised at the beginning of the paper, whether the measured values truly represent the quantum yield of the assimilatory apparatus, must for the present be answered in the negative, because the observed deviations from expected pressure change indicate departures of  $\gamma$  from unity. The manometric method, as it has been applied to the measurement of photosynthetic efficiency, is based, as we have explained, on the assumed constancy of y. In view of the apparent variability in y disclosed by our results, measurements made in this way cannot be accepted as significant until the method has been applied in such a way as to permit the simultaneous determination of both carbon dioxide and oxygen exchange. Yields higher than 0.25 may be due only to incorrect interpretation of measurements, the first alternative mentioned at the beginning of Part II. But correct interpretation of the measurements, based on determinations of the value of  $\gamma$ , may still result in quantum yields appreciably higher than 0.25. For this reason it seems necessary to consider the second alternative, that the chemical changes actually involved are different from those assumed to take place. As explained in the introduction to Part II, the theoretical maximum yield of 0.25 is based on the assumed synthesis of carbohydrate from carbonic acid.

The chemical mechanism of photosynthesis.—The commonly accepted value of unity for the ratio of exchange of carbon dioxide and oxygen (obtained as a rule from measurements of longer duration than

the efficiency measurements) has been regarded as evidence that intermediate substances less reduced than carbohydrate do not accumulate during photosynthesis. As a result of unsuccessful attempts to make *Chlorella* cells synthesize carbohydrates from possible intermediate substances, Warburg (1925, p. 402–403) believed that such substances probably played no part in photosynthesis. Probably for these reasons, Warburg and Negelein felt justified in regarding their measurements of efficiency as representing the complete synthesis of carbohydrate from carbon dioxide and water.

Our own observations, as set forth in the discussion of figure 2, are a clear indication that the ratio of exchange of carbon dioxide and oxygen departs from unity during the short light exposures used for efficiency measurements. If, as still remains to be shown, the deviations in y are indeed connected with photosynthesis, then it will be necessary to reconsider the possible rôle of intermediate products. The departure from unity of the value of y for photosynthesis suggests the temporary accumulation or disappearance of substances less reduced than carbohydrate. If during short light exposures carbon dioxide were only partially reduced, or if carbohydrate were formed from partially oxidized intermediates of respiration (a possibility recognized by Warburg, 1920, p. 205), then large deviations in  $\gamma$ would be anticipated. Such syntheses might require appreciably less energy than complete synthesis of carbohydrate from carbonic acid. Yields as high as are indicated by our present figures may be quite consistent with reaction mechanisms of this type. The efficiency measured over short intervals may considerably exceed the theoretical maximum for complete carbohydrate synthesis, which presumably becomes the dominant process only after a period of illumination sufficiently prolonged so that photosynthesis has reached a stationary state, and all steps in the process are proceeding at the same rate.

Part III. Experimental details.—The technique of culturing.—The organism used was Chlorella pyrenoidosa, isolated in 1926 from Berlin tap water and maintained since then on mineral agar slants exposed to daylight at a north window. A stock suspension of cells was obtained by inoculating a liquid culture with a mass of cells transferred from an agar slant with a needle. This liquid culture

was then grown at a temperature of about 20° with continuous illumination from a tungsten filament lamp (100-watt bulb at 20 cm.). After five to ten days' growth, when the culture had attained a moderate density, the concentration of cells was determined by centrifuging a sample into a calibrated capillary tube. The stock suspension usually contained from one to two mm.<sup>3</sup> cells per ml. of fluid. It was stored in the refrigerator and used for the inoculation of liquid cultures in which material for the efficiency measurements was grown. When exhausted, the stock suspension was replaced by another, again produced by growing a liquid culture inoculated directly from an agar slant.

The liquid cultures were grown in flasks of the usual pattern. These were made from 300 ml. Erlenmeyer flasks, by sealing inlet and outlet tubes for gas circulation into the sides of the necks. The tops, instead of being sealed over, were left open for greater convenience in filling and inoculation. The flasks were filled with the medium (200 ml. per flask), plugged with cotton, sterilized, and inoculated from the stock suspension, 5 ml. of stock being transferred to each culture with a sterile pipette. The cotton-plugged tops were then covered with tight-fitting rubber caps, so that a series of flasks could be connected to a single gas stream in the usual way.

The inoculated flasks were stored in the refrigerator and set out to grow in the illuminated culture baths as frequently as material was required for experimental work. Storage of the stock suspension or of the ungrown cultures in the refrigerator for periods of several weeks seemed to have no effect on the metabolism of the cultures after reaching maturity. The mature cultures, however, could not be stored in the refrigerator, even for a few hours, without suffering a decrease in photosynthetic efficiency, although storage up to several days has been found to be without effect on the maximum rate of photosynthesis.

During growth the culture flasks stood in a stream of running water in shallow glass dishes accommodating four flasks each. The desired temperature, usually 20°, was maintained within about 2°. Illumination 24 hours a day was provided by internally frosted tungsten filament lamps located below the glass dishes. For most of the work described in this paper, 100-watt lamps were used without reflectors, and the intensity of illumination was varied by changing the distance of the lamp, rather than the watt rating. One lamp was used for each dish of four culture flasks. The distances specified in the text are from the top of the lamp to the bottoms of the culture flasks. The cultures were aerated with a slow stream of 5 per cent carbon dioxide in air. The cells settled slowly to the bottom of the flasks, and were stirred up by gentle shaking twice daily.

The culture medium was of the following composition:  $MgSO_4$ , 0.0100 molar;  $KNO_3$ , 0.0125 molar;  $KH_2PO_4$ , 0.0090 molar;  $Fe_2(SO_4)_3$ , 0.000005 molar or 0.000025 molar;  $CaCO_3$ , 0.00001 molar.

The stock solutions were stored in Pyrex bottles cleaned with hydrochloric acid and rinsed only with glass distilled water. Merck's reagent grade chemicals were dissolved in glass distilled water. This was obtained by redistilling the laboratory distilled water, which was from a tinned still, in an all-Pyrex still designed to give good counter current washing of the ascending vapor.

Stock solutions: A. MgSO<sub>4</sub>7H<sub>2</sub>O, 50 g./liter; B. KNO<sub>3</sub>, 25 g./liter; C. KH<sub>2</sub>PO<sub>4</sub>, 25 g./liter; D. Fe<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub>, 4 g./liter; E. CaCO<sub>3</sub> (suspension), I. g./liter

To prepare culture medium, 50 ml. each of stocks A, B, and C were mixed and made up to one liter with the desired kind of water. Then either 0.5 or 2.5 ml. of solution D were added, depending on the iron concentration desired. The calcium carbonate suspension was shaken and one ml. added per liter of medium.

Under some conditions the efficiency was found to be higher in media containing calcium carbonate, so it was regularly included in the medium, although no experiments were made to show whether the improved efficiency was due to the calcium or to impurities. Warburg and Negelein added no calcium because it was present in the tap water they used to prepare the medium. Rieke, using distilled water, specified the addition of calcium nitrate. But Chlorella, unlike higher algae, seems to require no calcium for normal growth (cf. Pringsheim, 1926; Trelease and Selsam, 1939), and its presence may not be necessary for high efficiency of photosynthesis.

The filling of the manometer vessels.—To prepare cells for measurement of efficiency, they were centrifuged out of the culture medium, washed twice in freshly prepared medium, and made up to 28 ml. in medium saturated with 5 per cent carbon dioxide in air. Three ml. were used to determine the concentration of cells in the suspension, by centrifuging to constant volume in a calibrated capillary tube. The remaining 25 ml. were pipetted into the manometer vessel, and 3 ml. of medium added, bringing the total fluid volume to 28 ml. Ordinarily, medium with high iron concentration prepared in glass distilled water was used as suspending fluid for the experimental work, regardless of the medium in which the cells had been grown. However, in cases where higher concentrations of the major nutrient salts had been used for culturing the cells, the same concentrations were used for the measurements of photosynthesis, to avoid subjecting the cells to changes in osmotic pressure.

The manometer and vessels were similar to the apparatus described and illustrated by Warburg (1926, p. 1). After the vessels had been filled and connected with the manometer, the gas spaces were flushed out with 5 per cent carbon dioxide in air. The vessel containing the cells was then covered with a jacket of thin sheet brass with aluminum lining to reflect back into the cell suspension any light scattered out at the sides. This replaced the silvering and copper plating used by Warburg and Negelein.

A circular hole in the bottom of the jacket exposed almost the entire area of the bottom of the vessel to light, and served to define the area of cross section of the light beam admitted, which was 15.4 cm.<sup>2</sup>.

The measurement of photosynthesis.—For making the measurements, the vessels were submerged in a large water bath the temperature of which was maintained constant within a few hundredths of a degree, and the manometer yoke was connected to the shaking apparatus, an eccentric ball, the speed of rotation and eccentricity of which could be varied over a considerable range. Rapid mixing of the cell suspension is important. We found that an eccentricity of 4.3 mm. and a speed of 550 r.p.m. gave satisfactory results. Mixing was greatly improved by the use of a paddle-like extension of the vessel stopper, which projected downward at an angle into the suspension near the side of the vessel, adding turbulence to the rapid rotary motion of the liquid.

Pressure changes were measured without interrupting the shaking of the manometer and vessels, by reading the level of the manometer fluid (isocaproic acid) in both arms of the differential manometer simultaneously with a double cathetometer reading in hundredths of a millimeter. Random fluctuations in the readings varied from one or two hundredths of a millimeter to as much as one-tenth of a millimeter. There were occasional greater irregularities in the pressure changes, some of which seemed to be caused by slight changes in the temperature of the manometer fluid. These were minimized by protecting the manometer from air currents with a glassfronted box through which readings were made. Other irregularities appeared to originate inside the vessel, possibly from the random formation and breaking of bubbles in the agitated cell suspension. In our experience, the use of paraffin oil, recommended by Warburg and Negelein for the control of bubbles, has been of little or no value, so we omitted it in most of our work. The good agreement of the efficiency from successive light exposures showed that random irregularities in the pressure readings caused no appreciable error. The first measurement was always discarded, and the average photosynthesis from the next two or more light exposures was taken as the final value.

Light source and optical system.—A 10,000-lumen sodium vapor lamp (type Na9, obtained from the General Electric Vapor Lamp Co.) was used as a source of illumination. By enclosing the lamp in a wooden housing and running it above normal operating temperature, the light from the central region of the lamp was kept very uniform in intensity and practically free from neon lines. The infrared was removed by a 4.5 cm. layer of 1.65 per cent copper sulfate. This also served to reduce the very small amount of visible red neon radiation. Wave lengths shorter than the sodium line were of negligible intensity, but were sometimes removed by a yellow glass filter (Corning "yellow-yellow," No. 351).

A condenser was illuminated with the central area of the lamp. An image of this lens was projected by

a second lens and reflected vertically through a glass window in the water bath onto the vessel containing the cell suspension. The maximum available intensity was about 6500 ergs/cm.<sup>2</sup>/sec. This could be reduced by introducing calibrated diaphragms at the second lens. Photosynthesis measurements could be easily made down to 200 ergs/cm.<sup>2</sup>/sec.

Measurement of input radiation.—The energy was measured by projecting the beam of light horizontally onto a measuring instrument (bolometer, thermopile, or photronic cell) instead of into the cell suspension. This was done without disturbing the train of lenses and filters, by rotating about the axis of the beam the 45° mirror which served to reflect the light into the vessel. To maintain the similarity of the two optical paths, a window similar to the multiple window of the tank was placed in front of the measuring instrument. There remained the difference in the paths due to the layer of water and the vessel window, for which correction was made by multiplying the measured energy by 1.028. The bolometer diaphragm admitted a light meam 21 by 22 mm. in cross section. The surface of the thermopile was 1 × 10 mm. These instruments were calibrated against radiation standards from the National Bureau of Standards. The latter were operated on direct current and controlled with the help of a potentiometer and standard cell, by which both the current and voltage could be measured. The potentiometer was also used to measure the voltage applied to the bolometer and the compensating e.m.f. with which its circuit was balanced. The Weston photronic cell was mounted at the back of a small box with white inside walls. Light was admitted to the box through an opal glass window opposite the photocell, with a diaphragm equal in area to the one admitting light to the cell suspension. To insure linearity of response, the resistance of the electric circuit was kept down to 5 ohms. This combination was calibrated against the bolometer and thermopile in uniform parallel light from the sodium lamp. The three instruments gave results agreeing in most cases within a per cent at the lower intensities. At the higher intensities the beam was too sharply convergent for accurate measurement except by the photronic cell.

Intensity measurements were also made with the photronic cell placed in the water bath, in the same position occupied by the cell suspension. After proper corrections were made for reflection losses, these measurements were in good agreement with those made with the measuring instruments outside the tank. A few efficiency measurements were made with a beam of small enough cross section so that both the vessel and the measuring instrument could admit the entire beam. The results were in agreement with the other system of illumination.

Correction for incomplete light absorption.—Ordinarily efficiency measurements were made with a suspension of cells sufficiently dense to absorb all

the light entering the vessel, so that no provision was made for accurate measurement of the light transmitted. However, in measuring the dependence of the efficiency upon the culture medium, as in tables 1 and 2, it was considered desirable to harvest the cultures as nearly as possible at the same age. In several cases this resulted in cultures which failed to provide enough cells to give complete absorption of the incident light. By observing visually the light transmitted by the suspension during agitation in position for measurement of photosynthesis, a rough estimate was made of the percentage absorption. This percentage of the measured incident radiation was used in computing the efficiency. The estimated absorption for each culture is given in the tables as an indication of the error that may have arisen from this source in the determination of the efficiency.

Under standardized conditions, such as optical system and intensity, it was found that independent absorption estimates by two observers agreed within 5 per cent on an arbitrary scale. This scale was fixed partly by observing the beam of sodium light through neutral filters of known transmission and by making allowance for scattering by the cell suspension. The accuracy of our arbitrary scale of absorption was tested by measuring efficiency with three concentrations of similar cells, which were estimated to absorb 85, 98, and 100 per cent of the incident light. The resulting efficiencies, corrected for these absorption values, were 0.322, 0.325, and 0.322. This agreement, though doubtless partly fortuitous, indicates that our arbitrary scale was fairly accurate. If we allow the possibility of an error of one third in our estimate of the transmitted light, then the corresponding uncertainty in the absorption would be 6 per cent for an absorption of 85 per cent, and 11 per cent for an absorption of 75 per cent. In the majority of our efficiency measurements this error was negligible, since the estimated absorption was between 95 and 100 per cent. Admitting the unsatisfactory character of visual estimations of light absorption, we feel confident that the error introduced in this way is not sufficient to alter the conclusions drawn from the various tables.

Computation of photosynthesis and efficiency.—
The amount of carbon dioxide assimilated was computed by multiplying the pressure change due to photosynthesis by a factor designated as the vessel constant. The derivation of this constant is explained by Warburg (1926, p. 8–11, 114). We give below the necessary data for the computation of one of our own vessel constants.

 $P_{o} = 11,180$  mm., normal pressure in mm. of isocaproic acid.

T = 283°K, temperature of vessels.

v<sub>G</sub> = 17,130 mm.3, gas volume in experimental vessel.

v<sub>F</sub> = 28,000 mm.3, fluid volume in experimental vessel.

 $v'_{G} = 16,490$  mm.3, gas volume in compensation vessel.

 $v'_{E} = 28,000 \text{ mm.}^3$ , fluid volume in compensation vessel.

A=0.265 mm.2, area of cross section of manometer capillary.

 $a_{\text{CO}_2} = 1.188$ , Bunsen absorption coefficient for carbon dioxide in the liquid medium.

 $\alpha_{00} = 0.038$ , Bunsen absorption coefficient for oxygen.

 $\alpha = 0.06$ , Bunsen absorption coefficient for the gas in the compensation vessel.

 $\gamma = -0.9$ , ratio of exchange,  $CO_2/O_2$ , in photosynthesis.

By the formula given by Warburg, these values lead to  $k_{O_2}=1.838$  and  $k_{CO_2}=4.953$ . The vessel constant for oxygen is then  $k_{CO_2}\,k_{O_2}/(k_{CO_2}+\gamma\,k_{O_2})=2.76$ . The oxygen is used as a measure of the amount of carbon dioxide reduced, on the basis of considerations explained in detail by Warburg and Negelein (1922, part III).

Certain of these quantities call for some comment. The volumes of the vessels and connecting gas spaces were determined by filling them with mercury and weighing the mercury. Successive determinations agreed within about one hundredth of a per cent.

The value of  $a_{CO_2}$ , the Bunsen absorption coefficient for carbon dioxide, may not be the same for the fluid used in our vessels as the tabulated value for pure water. Warburg made a correction for this on the basis of measurements of Geffken. We made measurements of  $\alpha_{CO_2}$ , both for the pure water and for the culture medium used as suspending fluid. We were able to check the tabulated values for pure water at 0°, 8°, and 22° within about 1 per cent. Our values for culture medium, measured at the same temperatures, were between 0.8 and 2.0 per cent lower than for pure water, the same order of magnitude as the 1 per cent correction used by Warburg and Negelein. Since the salt concentration of our medium was half as great as theirs, we thought it justifiable to use a smaller correction factor for  $\alpha_{\text{CO}_2}$  and made a uniform correction of 0.5 per cent in the tabulated values at all temperatures (Landolt-Boernstein, 1912, p. 599). This correction slightly lowers the computed efficiencies.

For  $\gamma$ , the ratio  $\mathrm{CO_2/O_2}$ , we have used —0.9. As explained in Part II, there are reasons for questioning the constancy of  $\gamma$  over the short periods used for measuring efficiency. But since we have as yet no information from which a more correct value could be derived, it seems justifiable to adhere to their figure, if only to keep our results comparable with theirs.

Sample experiment.—The efficiency at low intensity for the sixth culture in table 3 was calculated from the following readings, which are differences in millimeters between the levels in the two arms of the manometer. The times are designated by letters which correspond to those in figure 2. The first cycle of light and dark is omitted.

Light	t Time		Reading	Difference
	A	2:55	15.83 լ	4.10
On	В	3:00	19.93 ₹	1.10
	$\mathbf{C}$	3:05	22.10	7.58
Off	D	3:10	23.96	1.00
	$\mathbf{A}$	3:15	27.51	0.00
On	В	3:20	31.49	3.98
	C	3:25	33.52	<b>*</b> 0.0
Off	D	3:30	33.41	7.36
	$\mathbf{A}$	3:35	ر 38.85	4.00
	В	3:40	42.87	4.02
	$\mathbf{E}$	3:45	46.73	3.71
	F	3:50	50.44	3.11

Computation of the pressure change due to photosynthesis by Warburg's method, described in Part II, was made as follows:

	Second cycle	Third cycle
5 min. dark A-B	4.10	3.98
5 min. dark A-B	3.98	4.02
5 min. dark, average	4.04	4.00
15 min. dark	12.12	12.00
10 min. light, 5 min. dark	7.58	7.36
Difference	4.54	4.64
Average pressure change due to 10 min. light	4.59	mm.

The vessel constant for oxygen in this case was 2.691 mm.  $^3/\text{mm}$ . Therefore the oxygen produced due to 10 minutes of light was  $4.59 \times 2.691 = 12.35 \, \text{mm}$ .  $^3$   $O_2$ , or  $12.35 \times 6/22.4 \times 10^6 = 3.31 \times 10^{-6}$  moles  $O_2/\text{hr}$ . Each mole of oxygen is assumed to represent the reduction of one mole of carbon dioxide. From the heat of combustion of glucose, the energy stored was  $3.31 \times 10^{-6}$  moles  $CO_2/\text{hr}$ .  $\times$  112,000 cal./mole  $CO_2 = 0.371 \, \text{cal./hr}$ .

The light intensity was measured with the photronic cell, which gave the following galvanometer deflections: 28.8 mm. at 2:55, 28.4 mm. at 3:15, 28.3 mm. at 3:35. Calibration of the photronic cell gave 12.56 ergs/cm.²/sec. per millimeter deflection, so that 28.4 mm. corresponds to an intensity of 357 ergs/cm.²/sec. The area of the vessel diaphragm was 15.4 cm.², and the window correction was + 2.8 per cent, so that 5650 ergs/sec. entered the vessel. Since the estimated light absorption in this case was 98 per cent, the radiant energy absorbed by the cells

was 5530 ergs/sec. One mole quantum, or einstein, at the wave length used ( $\lambda = 589.2 \text{ m}\mu$ ) is equivalent to Nh $\nu = 2.021 \times 10^{12}$  ergs. The energy absorbed was therefore 5530 ergs/sec. = 0.476 cal./hr. =  $9.85 \times 10^{-6}$  mole quanta/hr.

From the above figures, the efficiency of photosynthesis may be expressed as 0.371 cal./hr. stored  $\div$  0.476 cal./hr. absorbed = 78.0 per cent, or as  $3.31 \times 10^{-6}$  moles CO<sub>2</sub> reduced per hr.  $\div$  9.85  $\times$  10<sup>-6</sup> mole quanta absorbed per hr. = 0.336 (moles CO<sub>2</sub>/mole quantum) = 0.336 (molecules CO<sub>2</sub>/quantum).

#### SUMMARY

Using the manometric method developed by Warburg and Negelein, we have studied the dependence of the quantum yield of photosynthesis in *Chlorella pyrenoidosa* on various factors. High yields are favored by the presence of traces of certain heavy metals in the culture medium. The conditions during growth of cultures, and during measurement of photosynthesis, also influence the apparent quantum yield. With favorable combinations of conditions, yields as high as 0.33 molecule of carbon dioxide per absorbed quantum have been obtained, with indications that still higher efficiencies are possible.

A yield of 0.25 has heretofore been regarded as close to the theoretical maximum, because it has been believed that photosynthesis represents the complete synthesis of carbohydrate from carbon dioxide and water. If appreciably higher yields are obtainable, we must consider the possibility that some less complete synthesis may take place. This would involve a change in the usual ratio of unity for the exchange of carbon dioxide and oxygen. The behavior of the pressure change observed during the short periods of light and darkness used for measuring efficiency indicates that there are large variations in the ratio of exchange of oxygen and carbon dioxide.

Since the computation of photosynthesis from measurements of pressure change is based on the assumed constancy of this ratio, we conclude that the efficiencies obtained from the usual manometric measurements are subject to certain errors and may not represent the true efficiency of the assimilatory apparatus.

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# NATURE OF GROWTH DIFFERENCES IN TWO SORGHUM VARIETIES. I. INFLUENCE OF PRELIMINARY SOAKING ON EARLY GROWTH AND AUXIN CONTENT 1

# Robertson Pratt and Harry G. Albaum

The study of physiological differences among varieties of a single species is not only an interesting field of investigation but it may yield important information concerning the mechanisms of different processes. The present paper is the first of a proposed series dealing with differences in two varieties of Sorghum vulgare—namely, Standard Yellow Milo and Dawn Kafir. Experiments performed independently by the authors revealed striking differences in the early growth rates of these two varieties, seedlings of the former grown in the dark developing much more rapidly and being much sturdier than those of the latter. The results of the experiments suggested a hormonal interpretation and led to the work reported herewith.

Methods.—Pure strains of Sorghum vulgare (varieties Standard Yellow Milo and Dawn Kafir) were used throughout these experiments.<sup>2</sup> All water used in the growth experiments was distilled twice, the second distillation being carried out entirely in Pyrex apparatus.

In each experiment, unless otherwise indicated, groups of 35 seeds were soaked in 50 cc. of distilled water, each lot of seeds being transferred to fresh 50 cc. portions of water hourly for six hours, and then every twelve hours, as long as soaking continued. After soaking they were placed, embryo side up, on three layers of moistened filter paper in Pyrex Petri dishes. When the roots were about 5 mm. long, 25 representative seedlings were transferred with clean forceps to perforated cork discs previously coated with paraffin. The roots protruded through the holes and into the distilled water on which the corks floated, each in a separate beaker. The seed-

<sup>1</sup> Received for publication September 20, 1939.

<sup>2</sup> Seeds were kindly furnished by Dr. G. M. Reed and Dr. D. E. Marcy of the Brooklyn Botanic Garden.

lings were carefully removed at suitable intervals, root and shoot lengths were measured with a millimeter scale, and the seedlings were replaced on the discs. Thus, measurements were obtained on the same sets of seedlings throughout each experiment. The plants were in the dark at all times except while the water was changed and measurements were made, at which times they were exposed to weak, diffuse light from a Mazda lamp.

Some of the quantitative differences in the growth experiments may have been due to fluctuations in the temperature, which varied from 18°-25°C. The general trend was the same in all experiments, however.

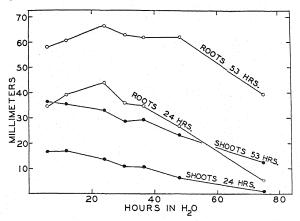
It is improbable that the effects observed in these experiments were due to poor aeration, because the results were essentially the same when the water was continuously aerated while the seeds were immersed in it. Furthermore, germination, as indicated by the initial protrusion of primary roots, occurred readily in completely submerged seeds of both varieties.

RESULTS.—The experimental procedure seemed to exert little, if any, effect on the growth of Dawn Kafir seedlings but markedly influenced the growth of the Yellow Milo variety.

The pronounced effect on Yellow Milo seedlings is shown in figure 1, in which data from a typical experiment are presented. The mean length of roots and shoots (internodes plus coleoptiles) at different times is plotted on the ordinate scale and the length of the soaking period on the abscissa. The shoot length was always inversely related to the duration of the previous period of soaking. Root growth, on the other hand, increased as the period of soaking increased up to 24 hours but was retarded by longer immersion.

The results suggest that when Milo seeds are soaked in water a substance that may be tentatively identified as an auxin or its precursor leaches out of the seeds or is inactivated or destroyed. Data presented below indicate that the observed effect was probably due in large measure to auxin's being leached out of the seed.

Experiments have indicated that, in some plants at least, the optimum auxin concentration for root growth is lower than for shoot growth (Thimann,



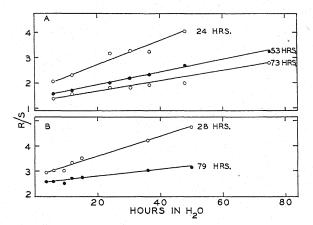


Fig. 1 (above). Mean length of roots and shoots of Sorghum vulgare var. Yellow Milo at different times after different initial periods of soaking in water.

Fig. 2 (below). Mean length of root in relation to that of shoot (R/S) in seedlings of  $Sorghum\ vulgare\ var.$  Yellow Milo at different times after different initial periods of soaking in water.

1937; Kaiser and Albaum, 1939). Removal of some of the auxin, or its precursor, might therefore be expected, under certain conditions, to retard the growth of shoots relatively more than that of roots; and, indeed, it is readily conceivable that extraction of some of the hormone would bring about an increase in the growth of roots while causing a decrease in shoot growth. The data presented by the curves in figure 1 are in agreement with this hypothesis and may be interpreted to mean that during the

soaking period an auxin or some related compound diffused out of Yellow Milo seeds and that after approximately 24 hours the supply within the seeds was reduced to the optimum level for root growth under the experimental conditions. They also seem to indicate that with further soaking, the amount of auxin present in the seed was reduced to such a low level that it became limiting for root growth as well as for shoot growth. Longer periods of soaking, therefore, brought about a decrease in the length of both organs. According to this idea, the amount of auxin initially present was sub-optimal for the growth of shoots. Therefore, the size of these organs decreased even with the shortest periods of immersion.

The failure of Dawn Kafir seedlings to respond to the experimental treatment may indicate that it was impossible under the conditions of these experiments to reduce the hormone content sufficiently to cause any appreciable change in growth, either because the seed coats of this variety are relatively impermeable to auxins or because there is a high degree of auxinsatiety.

Figure 2 shows the mean length of the primary root in relation to that of the shoot (R/S ratio) in Yellow Milo seedlings at different times after different periods of soaking. The two upper curves in figure 2A are derived from the data in figure 1; those in figure 2B represent the averages obtained from three later experiments. At different times during the course of the experiments, the value obtained when the mean length of the primary roots was divided by that of the shoots increased with the length of time the seeds had previously been soaked. Although the points are somewhat scattered, the curves seem to be approximately linear over the period of time covered in these experiments. That the effect of soaking the seeds was most pronounced in the very early stages of development is shown by the higher levels and greater slopes of the curves for shorter periods of growth. The important point for the present discussion is that in any one curve the root/shoot ratio increases with the length of the previous soaking period. This indicates that shoot growth was retarded relatively more than rootgrowth as the preliminary period was extended.

Table 1. Influence of soaking seeds in water on the subsequent root/shoot (R/S) ratios in Yellow Milo and Dawn Kafir seedlings grown in distilled water in the dark.

Hours	R/S at	R/S at 24 hrs.a		53 hrs.a	R/S at 73 hrs.a	
soaked	Milo	Kafir	Milo	Kafir	Milo	Kafir
6.0	2.08	3.63	1.59	2.43	1.35	2.17
12.0	2.29	3.68	1.70	2.47	1.58	2.21
24.0	3.17	4.86	1.98	2.95	1.84	2.61
30.5	3.28	3.80	2.18	2.22	1.82	1.82
36.5	3.22	3.65	2.09	2.07	1.92	1.90
48.0	4.05	3.17	2.68	1.93	2.00	1.73
75.0	5.35	3.27	3.22	2.80	2.80	2.24

a Hours from the time seedlings were placed on corks.

Data from a typical experiment are presented in table 1 and clearly demonstrate the pronounced difference in the effect of soaking on the two varieties of seeds. Note that while the values for Milo steadily rise in each column, those for Kafir show no consistent tendency to either rise or fall, indicating that roots and shoots were about equally affected or were not affected at all by the soaking treatment.

Results of other experiments also support the hypothesis that the response of Yellow Milo seedlings to prolonged soaking is due, in part at least, to partial depletion of the supply of an auxin or some related compound.

If the effects recorded above were due to loss of an auxin, frequent changing of the water in which the seeds were soaked would be expected to facilitate its removal, and prolonged soaking in a very small volume of water that is not changed might be expected to cause relatively less decrease in shoot growth and relatively greater decrease in root growth than soaking in an equal volume of water with numerous changes. In other words, frequently changing the water in which the seeds are soaked should tend to increase the root/shoot values. Accordingly, the following experiment was performed.

A group of fifty Milo seeds was placed in each of two small vials, and enough water (2.5 ml.) was added to just barely cover the grains.<sup>3</sup>

In one group ("C") the water was withdrawn by means of a pipette, and fresh water was added hourly for the first 8 hours, and then at 8-hour intervals for the next 40 hours. The water of the other group ("U") was not changed during the 48 hours that the seeds were soaked; as they swelled, suffi-

Table 2. Root and shoot lengths in Yellow Milo seedlings. See text for explanation.

	Water	changed	—"С"	Water no	ot change	d—"T
Hrs. of growth	Root length, mm.	Shoot length, mm.	R/S	Root length, mm.	Shoot length, mm.	R/S
24	26.5	7.80	3.40	24.5	10.31	2.37
24	28.3	7.08	4.00	24.8	9.30	2.67
24	25.2	6.72	3.75	26.1	8.50	3.07
Average	26.6	7.2	3.69	25.1	9.37	2.68

a Hours from the time seedlings were placed on corks.

cient water was added to keep them just barely submerged at all times. After 48 hours' immersion, both groups of seeds were placed on filter paper in the usual way and were transferred to corks 34 hours later. Nearly all seeds in both groups had germinated and had roots about 1 mm. long at the end of the soaking period. At that time about two-thirds of the seeds in the "U" series had shoots ½-1 mm. long. Shoots were not apparent on any seeds of the "C" series. This experiment was repeated twice, and the figures obtained are presented in table 2. It may be seen that the data are in good agreement with expectations based on the hypothesis outlined above.

In another experiment, one hundred seeds of each variety were soaked in 100 ml. of water for 40 hours. and after removal of the seeds the water from each group was evaporated under reduced pressure at 65-70°C. to approximately 1 ml. These 1 ml. samples were then thoroughly mixed with equal portions of lanolin, and the resulting pastes were applied to the upper surfaces of petioles of young leaves of Atropa belladonna. Twelve hours later epinastic curvatures had resulted in 6 of 12 petioles treated with Milo extract, but none of those treated with Kafir extract had responded. This experiment was not repeated, and it is not entirely satisfactory, since it does not tell whether the lower auxin content (or its absence) in the Kafir extract was due to impermeability of the seed coats or to a smaller amount of auxin present in the seeds.

It seemed desirable to determine the relative amount of auxins in each variety of seed after treatment similar to that used in the growth experiments. Accordingly, different lots of seeds of the two varieties were soaked in small quantities of water for 30 hours, the water of one group of seeds ("C") of each variety being changed hourly for the first 8 hours and then less frequently and that of the other group ("U") remaining unchanged during the entire period of soaking.

The seeds were then extracted with chloroform according to the method of Thimann (1934), and the extracts were tested for the presence of auxins by means of the usual Avena-coleoptile curvature method. The oat variety, Victory, was used for these tests, and the double decapitation method described by Went and Thimann (1937) was employed. Ten to twenty test plants were used in each experiment, and shadowgraphs were made 90 minutes after application of the agar blocks.

Table 3. Avena curvatures induced by different Sorghum extracts. See text for explanation.

	Av. cur	vature		Av. cui	rvature	
Number	Milo, unchanged	Milo, changed	M. C./M. U.	Kafir, unchanged	Kafir, changed	K.C./K. U.
1	10.9°	7.9°	0.72	20.5°	18.2°	0.89
2	13.0°	9.0°	0.69	20.0°	18.1°	0.91
3	6.2°	4.5°	0.72	10.7°	9.3°	0.87

<sup>&</sup>lt;sup>a</sup> Twenty-five seeds extracted for each series.

<sup>&</sup>lt;sup>3</sup> Since Kafir seeds were not responsive to the soaking treatment, only Milo seeds were used.

The results of tests repeated on different days are shown in table 3. The test plants used in the third trial were about 24 hours older than those used in the first and second trials and were less sensitive. However, although the magnitude of response was reduced, the relative response to the extracts from the "unchanged" and "changed" series was not significantly different from the earlier tests. The data in table 3 seem to show rather clearly that the Kafir seeds are less permeable to auxins than Milo seeds, since frequently changing the water, presumably maintaining thereby a steep concentration gradient from internal to external solution, caused greater loss of auxin from Milo than from Kafir seeds. It should be borne in mind that extremely small volumes of water were employed. Similar results probably could not be so easily detected with large volumes of water, since it is doubtful that the hormone concentration in the external medium would become sufficiently great to markedly inhibit its outward diffusion. Column 4 shows that approximately 70 per cent as much auxin was present in Milo seeds when the water was frequently changed as when it remained unchanged, but the corresponding value for Kafir seeds was about 90 per cent (column 7).

It is apparent from the figures in table 3 that frequently changing the water in which the seeds were soaked caused relatively greater loss of auxin from Milo seeds than from Kafir and that after the soaking period, whether the water was changed or unchanged during the period of immersion, Kafir seeds contained more of the compound than did Milo. It should be noted, however, that in the dry condition, before soaking, seeds of the two varieties contained approximately equal quantities of the hormone (table 4, column 3).

Comparison of tables 3 and 4 shows that the auxin content of Kafir seeds increased considerably during the soaking period, and although the available data are not adequate to tell whether or not there was an increase in Milo seeds also, it seems reasonable to assume that the preliminary stages of germination would follow the same course in both varieties of *Sorghum*. Thus it appears likely that two factors are involved in these experiments—i.e., permeability of the seed coats to auxins and rate of

hormone production. The possibility should not be ignored, however, that the rate of auxin production may be the same in both varieties and that the apparent greater speed in Kafir seeds is merely due to the accumulation of hormone that cannot escape through the seed coat of this variety as readily as through that of Milo seeds. In order to learn about the total amount of auxin formed it would be necessary to assay not only the seeds, but also the water in which they were soaked. This was not done, except indirectly in the petiole-bending tests referred to above. It should also be pointed out that escape of auxin from seeds may depend upon its location within the seeds and that the proportion of auxin or its precursor in the embryo and endosperm, respectively, may influence their outward diffusion.

The data so far considered concern the amount of auxin present in the two varieties of seeds but tell nothing about its concentration, a factor which might be of considerable importance in determining growth rates. To obtain a true picture of the relative concentrations of auxins in the two varieties of seeds, it is necessary to make a correction in the observed figures because of the difference in size and weight, Milo seeds being larger and about 30 per cent heavier (air dry weight) than Kafir. The data for the "unchanged" seeds (M. U. and K. U.) in table 3 have been treated accordingly, and the corrected figures are given in table 5. The data in table 4 have also been treated in the same manner, and the corrected figures are shown in column 4.4 The last column in each table affords a measure of the rela-

Table 5. Avena curvature per gram extracted material.

Data from table 3. See text for explanation.

	Curvature		
Test number	M. U.	K. U.	M. U./K. U.
1	12.0°	29.5°	0.42
2	14.0°	28.8°	0.48
3	6.8°	15.4°	0.44

<sup>&</sup>lt;sup>4</sup> These corrections are only approximations, since the individual sets of seeds were not weighed. The weights of the different groups were estimated from the known weights of 500 seeds of each variety.

Table 4. Avena curvatures induced by extracts of dry Sorghum seeds. See text for explanation.

		Av. cu		
	No. test plants	Actual	Degrees/gm. dry wt.	M/K
Test No. 1:				
Dawn Kafir	21	9.6°	12.8)	
Yellow Milo	19	10.8°	10.4	0.81
Control 0.008 mg. 3-indole-acetic acid/l.	10	11.1°		
Test No. 2:				
Dawn Kafir	19	11.5°	15.4)	
Yellow Milo	17	11.5°	11.0	0.72
Control 0.008 mg. 3-indole-acetic acid/l.	10	13.1°		

a Twenty-five seeds extracted for each test.

tive auxin concentration (not total content) in the two varieties. It may be seen that in the dry condition, before soaking, the auxin concentration in Yellow Milo seeds was about 70-80 per cent as great as in Kafir seeds (table 4, last column) but that after soaking for 30 hours under the conditions of these experiments, the hormone concentration, as measured by degrees of curvature induced per gram of extracted material, was about 40-45 per cent as great in Milo seeds as in Kafir (table 5, column 4).

This enormous difference in hormone concentration may be an important factor in determining the rate of early growth of these two varieties of Sorghum. If this proves to be so, it will be an interesting example of the determining influence of natural differences in hormone concentration on the growth and development of plants. Van Overbeek (1935) has shown that in corn, stunting of the dwarf variety, Nana, may be due to a natural deficiency of available auxin, the supply in the plant being destroyed by enzymatic action. It is believed that the present case—i.e., the relatively puny early growth of Dawn Kafir seedlings, may represent a tendency in the other direction—i.e., a plant which grows slowly, because it contains a surplus of auxin, and is not able to destroy or eliminate the excess. Further experiments are being planned to test this hypothesis.

#### SUMMARY

Seeds of Sorghum vulgare, varieties Yellow Milo and Dawn Kafir, were soaked in distilled water for different lengths of time. After germination had occurred, they were placed on perforated corks arranged so that the roots dipped into distilled water, and the subsequent growth of roots and shoots (internodes plus coleoptiles) was observed for 2-3 days.

The growth of Dawn Kafir seedlings was not appreciably affected by the different treatments, but that of Yellow Milo was markedly altered.

The shoot length at any time was inversely related to the duration of the previous period of soaking. Root-growth, on the other hand, increased as the period of previous soaking increased up to 24 hours but was retarded by longer periods of immersion.

The ratio obtained when the mean root length at any time was divided by the mean shoot length at the

same time increased as the previous period of immersion increased, indicating that shoot growth was retarded relatively more than root growth.

The effects on growth were most pronounced in the very early stages of development.

Auxin-assays by means of the Avena-coleoptile method showed that the auxin content of dry seeds of the two varieties was essentially the same but that after the experimental treatment Kafir seeds contained considerably more hormone than Milo. Before and after soaking, the relative hormone concentrations (not total contents) in Milo seeds were about 75 and 45 per cent, respectively, of those in Kafir seeds. Three factors may be involved—i.e., rate of auxin formation, permeability of seed coats to the hormone, and distribution of the hormone between the endosperm and the embryo. The available evidence indicates that Dawn Kafir seeds are much less permeable to auxins than Yellow Milo seeds.

The experiments also showed that unmutilated, auxin-deficient seedlings may be easily produced experimentally. Such seedlings may be useful agents in investigations of the physiological rôle of auxins in plant cells.

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# THE EFFECT OF BORON IN THE SUBSTRATE ON CALCIUM ACCUMULATION BY SOYBEAN PLANTS <sup>1</sup>

C. E. Minarik and J. W. Shive

THE FIRST INVESTIGATORS to suggest an association between boron and calcium in plant metabolism were Brenchley and Warington (1927). Later work by Warington (1934) showed that the absence of boron from the nutrient solution depressed the amount of calcium absorbed by Vicia faba as compared with controls which received boron. A report by the Department of Plant Physiology, New Jersey Agricultural Experiment Station (1937) also suggests that boron may influence the normal metabolism of calcium in plants. Analyses of plant tissues by various workers (Bobko, Syvorotkin, and Filippov, 1939; Hill and Grant, 1935; Rehm, 1937) indicate that the addition of boron to the substrate increases the calcium content of the plants as compared with check plants receiving no additional boron. Other reports (Hill and Grant, 1935; Talibli, 1935) state that boron decreases the calcium content of plants as compared with controls. Several other investigators (Holley and Dulin, 1937; Morris, 1938) have found that the presence or absence of boron in the nutrient medium has no effect on the amount of calcium in the plant tissues which were analyzed.

These inconsistent results suggest that if boron actually does influence calcium metabolism its effect varies with the species of plant investigated and with the conditions under which the experimental

procedure is carried out.

The purpose of this investigation was to determine the effective range of boron concentrations for optimum growth and to study quantitatively the effect of boron on the calcium metabolism of representatives of the two great groups of plants, monocots and dicots, whose morphological structure and physiological behavior differ widely and whose boron requirements are notably dissimilar. The plants selected are soybeans, a representative of the dicots, and corn, a representative of the monocots. The present paper deals with the results obtained with soybeans. The data obtained with corn will be presented in another publication. A preliminary quantitative investigation of the rates of calcium absorption and accumulation by the corn plant as affected by variable concentration of boron in the substrate presents a picture which bears little resemblance to that obtained for soybeans described in the following pages.

EXPERIMENTAL METHODS.—For these experiments sand culture was employed. The sand used had been previously treated with five per cent hydrochloric acid and thoroughly washed. This was followed in the same manner with a five per cent solution of ammonium hydroxide in order to remove any substances which might furnish boron or any other nutrients to plants growing in the sand. This precau-

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tion is necessary since boron from unknown sources present in the experimental set-up would render the results obtained from such cultures of doubtful value. Sand subjected to the above treatment was tested and found to be inert as far as plant nutrients are concerned.

Seeds of the Harbinsoy variety were obtained from the Agronomy Department of the New Jersey Agricultural Experiment Station. They were selected for uniformity of size and shape, soaked in tap water for several minutes, and then transferred to moist blotters. When the seeds had germinated and the tap roots were about five centimeters long, uniform seedlings were transferred to nine inch glazed pots which were filled with sand previously treated as described. The glaze on the pots employed was free from boron as determined by test. Three plants were placed in each pot. Solution was applied to the surface of the sand by the continuous solution renewal method of Shive and Stahl (1927).

A four salt standard solution prepared with sodium nitrate, calcium chloride, primary potassium phosphate, and magnesium sulfate previously used and tested by Shive (1936) in boron studies was here employed. Iron and manganese were added at the rate of 1.0 ppm. and 0.5 ppm., respectively. Boron was added at fourteen different concentration levels varying from 0.0 ppm. to 10 ppm. Duplicate

cultures were grown.

In eighteen days boron toxicity symptoms appeared in the leaves of the plants receiving 10 ppm. of boron. Necrotic spots also appeared on the leaves of the plants receiving low concentrations of boron. In the latter case, however, the spotting was due to iron toxicity, for when iron was withheld from the plants with spotted leaves the new leaves that were produced were normal. Iron was withheld for twelve days. When iron treatment was resumed, it was added at the rate of 0.25 ppm. to all cultures. Manganese was reduced from 0.5 ppm. to 0.25 ppm. since the reduced amount was found to be just as effective for the growth of soybeans as was the higher concentration. The plants were grown for seventyeight days before being harvested. At this time they had produced seed pods but the pods were not mature. At harvest the plants were fractionated into leaf blades, stems and petioles and seed pods. The fractions from the three plants in each culture were combined and weighed together. The tissue was then cut into small pieces, thoroughly mixed and aliquots were taken for dry weight determinations. The material was dried in an oven at 75°C. and then stored in air-tight bottles. Calcium determinations were made on this tissue.

CHEMICAL METHODS.—The official micro method of the Association of Official Agricultural Chemists (1935) with a few minor changes was used for the calcium determinations. The official method uses a

two gram sample of plant tissue ignited at a dull red heat (600°C.). Since insufficient material was available, a one gram sample was used in these studies.

The solution of the ash was made to volume in a 50 ml. volumetric flask. Five milliliter aliquots of this solution were used in each determination without previous removal of silicon dioxide. In the official method the silicon dioxide is removed just before the calcium is precipitated. Although silicon dioxide is coprecipitated with calcium oxalate, it does not interfere in the titration of the oxalate ions by permanganate ions. It is not essential, therefore, that it be removed. Coprecipitation of silicon dioxide would cause a serious error if the calcium were determined gravimetrically, but it is without effect in the procedure employed.

The remainder of the procedure used was the same as the official method, except that after centrifuging the calcium oxalate, the supernatant liquid was decanted rather than removed by suction. Tisdall (1923) has shown that decantation gives just as accurate results and is much more rapid than the suction method.

The oxalate ion was titrated with 0.01 n. KMnO<sub>4</sub> instead of the 0.02 N. solution which is recommended. This change was made in order to obtain greater accuracy. The 0.01 N. KMnO<sub>4</sub> was made up fresh each day by diluting a stock solution of 0.1 N. KMnO<sub>4</sub>. The stock solution was restandardized against sodium oxalate once a week.

EXPERIMENTAL RESULTS AND DISCUSSION.—Soybean plants receiving concentrations of boron less than 0.025 ppm. were typical low boron plants. Growth was retarded, the apical meristems died, and while axillary buds developed, they were short lived. The leaves developed necrotic spots between the veins and remained small. Plants receiving between 0.025 ppm. and 1.0 ppm. of boron appeared to be normal healthy plants. The leaves were dark green and were free from necrotic spots. Plants receiving

2.5 ppm. of boron and higher concentrations showed toxicity symptoms. The old leaves were chlorotic, and necrotic spots appeared along the margins. In the later stages of boron toxicity the necrotic spots were not confined to the leaf margins but appeared over the entire leaf surface. All the leaves, young and old, became chlorotic and spotted. The plants receiving toxic amounts of boron did not attain the full height that the normal plants did, but they were not stunted so badly as the boron deficient plants.

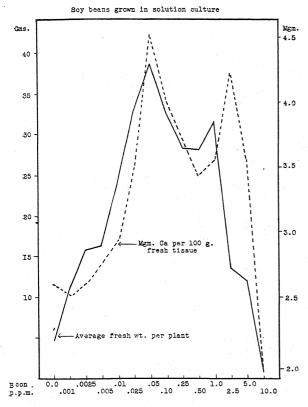
On the basis of the weight of fresh tissue produced per plant, as indicated by the data of table I, the optimum range of boron concentration for Harbinsoy soybeans grown under the conditions of these experiments lies between 0.025 ppm. and 1.0 ppm. Concentrations above and below these values were considerably less effective in the production of tissue than were the concentration in the optimum range.

Inasmuch as calcium generally accumulates to a greater extent in the leaves of a plant than in any other tissues, only the leaves were analyzed in this experiment. The calcium content of the leaf blades as well as the fresh weight per plant is roughly proportional to the amount of boron in the nutrient solution when sub-optimal quantities of boron are present in the substrate. When super-optimal quantities are present in the substrate, this relationship becomes an inverse rather than a direct one. This is clearly brought out by the comparative graphs of figure 1, in which the ordinates on the left represent the average weight of fresh leaf tissue per plant, and those on the right represent the average calcium content in milligrams per gram of fresh leaf tissue. Absissas indicate boron concentration of the substrate in parts per million.

When the concentration of boron in the nutrient solution was low enough to retard growth, the accumulation of calcium in the leaves was much lower than when optimum concentrations of boron were

Table 1. The average fresh weight, percentage moisture, calcium content and ash content of soybean leaves as influenced by boron.

Boron in substrate ppm.	Total fresh wt. of tops per plant in grams	Average fresh wt. of leaves per plant grams	Percentage moisture in fresh leaf tissue	Mgms. Ca per gm. fresh leaf tissue	Percentage ash in dry tissue	Percentage Ca in ash
0.0	10.3	4.7	81.4	2.6	13.7	10.4
0.001	24.1	11.0	82.0	2.5	12.6	11.3
0.0025	33.0	15.8	93.0	2.6	14.0	11.0
0.005	39.8	16.2	80.4	2.9	12.8	11.6
0.010	59.8	24.0	80.4	3.0	13.1	11.5
0.025	88.8	32.8	79.6	3.5	13.5	12.7
0.05	118.1	38.7	76.6	4.5	13.5	14.2
0.1	104.6	32.6	77.2	4.0	13.4	13.1
0.25	95.8	28.4	77.6	3.9	13.6	12.8
0.5	94.8	28.1	75.4	3.4	12.2	11.5
1.0	102.2	31.5	76.0	3.5	13.3	11.1
2.5	45.6	13.6	74.0	4.2	16.2	10.0
5.0	41.6	12.0	73.0	3.6	15.3	8.6
10.0	8.6	0.8	80.0	2.0	13.2	7.6



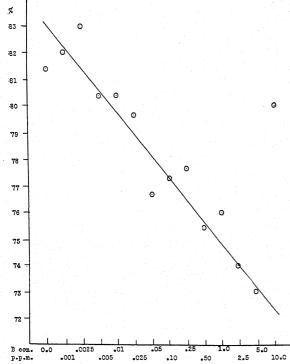


Fig. 1 (above). Graphs showing the average leaf yields and calcium content of leaf tissue in relation to boron concentration of the substrate.

Fig. 2 (below). Graph showing the effect of the boron concentration of the substrate on the moisture content of

present. When the concentration of boron was optimum for growth, the calcium content of the leaf blades was at a maximum. Concentrations which were toxic to the soybean plants reduced the amount of calcium accumulated as compared with plants receiving optimum quantities of boron. A marked deviation from this general rule occurred when the boron concentration was 2.5 ppm. Here the calcium content was quite high and the fresh weight per plant was quite low. No satisfactory explanation for this discrepancy can be made.

The graph representing the amount of fresh leaf tissue produced, as influenced by the concentration of boron in the substrate, closely parallels the graph representing the calcium content of the fresh leaf tissue. This similarity indicates a close correlation between tissue production and calcium content and shows that both are affected by boron in the same manner and to approximately the same degree. It is apparent from the data in table 1 and figure 1 that concentrations of boron above or below the optimum range produce plants with low calcium content as compared with plants in the optimum range of boron concentrations. This reduction in the calcium content below that of the plants in the optimum range may be sufficient to produce the symptoms generally associated with calcium deficiency. It is well known that the symptoms of calcium deficiency are rather similar to those produced by boron deficiency. Consequently, the symptoms of boron deficiency may be accompanied by those of calcium deficiency without easy detection. Calcium deficiency in such a case may be the indirect effect of boron deficiency, since the direct effect must be a change in the protoplasmic complex which makes it impossible for the plant to accumulate sufficient calcium for the maintenance of its normal metabolic activities.

Rehm (1937) working with Impatiens balsamina found that the addition of boron to the nutrient medium increased the intake of cations and retarded the intake of anions as compared with cultures lacking boron. Rehm suggests that boron unites with alcohols and sugars forming strongly acid compounds. This shifts the isoelectric points of the protoplasm to the acid side and favors the adsorption of cations and retards anion adsorption. This generalization is not substantiated by the work of Morris (1938), who found that the amount of calcium, potassium, and phosphorus in boron deficient oranges was no different from the amount present in normal fruit. Holley and Dulin (1937) likewise found no interrelation between boron and calcium or magnesium.

Talibli (1935) analyzed flax straw and found that the addition of boron to the substrate decreased the concentration of calcium, potassium, nitrogen, and phosphorus in the plants as compared with check plants. These results are not in line with Rehm's generalization.

The work of Rehm (1937) conclusively shows that his theory is applicable to Impatiens balsamina,

leaves. The ordinates indicate the percentage moisture and the abscissas the boron concentrations.

but apparently it does not apply to other species. The data in table 1 support the theory that calcium deficiency accompanies boron deficiency, but neither Rehm's theory nor the one presented here may be regarded as applicable to all plants.

In the case of plants receiving toxic quantities of boron the calcium content of the tissue is as low as that of plants receiving insufficient amounts of boron, yet here no symptoms which are usually associated with calcium deficiency are apparent. An explanation for this condition might be that a larger percentage of the total calcium present may be in an active form as compared with the percentage of active calcium in boron deficient plants.

Haas (1929) has shown that the calcium content of citrus and walnut leaves from plants which received toxic quantities of boron was less than the amount present in leaves from normal plants. However, the percentage of the total calcium that was water-soluble was higher in leaves of plants which received large quantities of boron than in the leaves of normal plants. Haas interpreted this to mean that the leaves showing toxicity symptoms did not mature, since the ash constituents of such leaves were present in the same proportions as they were in immature normal leaves. It may also mean that the calcium present is not precipitated and thus rendered inactive. Consequently, the small amounts which were present may have been sufficiently active to permit normal growth so far as calcium was con-

The data obtained with soybeans which are presented in table 1 agree with the results obtained by Haas with respect to the amount of calcium present in plants receiving toxic quantities of boron as compared with normal plants. The conclusion to be drawn from this is that quantities of boron which are toxic to soybean plants may prevent the normal accumulation of calcium in soybean leaves but that a large part of the accumulated calcium may remain water-soluble and may be sufficient to fulfill the requirement of the plant for this element.

Effect of boron on water content.—Besides influencing the tissue production and the calcium content of soybeans, boron also has an effect on the percentage moisture in fresh leaves. When the boron concentration of the nutrient solution was low, the water content of the soybean leaves was at a maximum. With rising concentrations of boron in the substrate the percentage moisture in the leaves decreased. The data in table 1 show this relationship. Figure 2 is a graphic representation of these data. The abscissas indicate the boron concentration of the nutrient solution, and the ordinates indicate the percentage moisture in the soybean leaves.

These results support Schmucker's (1935) hypothesis that boron exerts some control over the swelling of plasma colloids. Schmucker germinated pollen grains in sugar solutions with and without boron. Pollen grains in sugar plus boron germinated normally. Pollen in sugar solutions lacking boron burst shortly after the pollen tube emerged.

Schmucker concluded from this evidence that boron plays a rôle in the imbibition of water by the plasma colloids. In the absence of boron, imbibition of water is not regulated but continues until the cells burst. The presence of boron in the external medium appears to regulate the amount of water absorbed, perhaps through its influence upon the colloidal complex of the plant. The data of table 1 and figure 2 represent quantitative measurements which substantiate Schmucker's hypothesis. Boron, however, is not the only element whose absence from the growth medium produces a plant with a high moisture content. Calcium deficiency likewise increases the moisture content of plant tissues as compared with controls which received calcium. This fact further points to a metabolic relationship between the two elements in plant growth and offers a stronger basis for the suggestion that the absence of boron from the nutrient medium may produce calcium deficiency symptoms

CALCIUM CONTENT OF THE ASH .- The percentage of calcium in the ash of soybean leaves follows the same general trend that the fresh weight of leaves and the calcium content of the leaves follow. Although the percentage of ash expressed on the basis of dry tissue is not influenced significantly by the concentration of boron in the substrate, the percentage of calcium in the ash is much lower when concentrations of boron above or below the optimum range are used than when the optimum concentration of boron is present in the nutrient medium. This indicates that boron does not affect the percentage of ash in the dry matter but does influence the proportions in which the elements are present in the ash. When the percentage of calcium in the ash is low, the percentage of the other ash constituents is correspondingly high and vice versa.

#### SUMMARY

The production of fresh tissue by soybean plants as well as the percentage of calcium in the leaves of soybean plants is conditioned by the concentration of boron in the nutrient solution. Deficiency as well as toxicity quantities of boron in the substrate result in low yields and subnormal calcium in the tissues. The optimum range of boron concentrations in the substrate lies approximately between 0.025 ppm. and 1.0 ppm.

Within the range of concentrations here employed the percentage moisture in soybean plants decreases with increasing concentrations of boron in the nutrient solution. This supports the hypothesis that boron may be a regulator of water absorption by the plasma colloids.

The ash content of soybean leaves does not vary significantly with variations in the concentration of boron in the nutrient solution, but the calcium content of the ash is influenced by the boron content of the substrate, both excess and deficiency concentrations of boron in the substrate corresponding with low percentage values for calcium in the ash.

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# THE GENUS MASSARIOVALSA 1

# Lewis E. Wehmeyer

THE GENUS Massariovalsa was described by Saccardo (1882, p. 569) as a Massariella with perithecia arranged in a valsoid manner. The two-celled brown ascospores have a gelatinous envelope, and the asci are given as paraphysate. Apparently, only two species have been described in this genus, M. sudans (B. & C.) Sacc., the type species, and M. caudata E. & E. which the writer (1933) has shown appears under two descriptions, the first being a description of Pseudovalsa (Prosthecium) bicornis (Cke.) Sacc. and the second a description of a good species, Pseudovalsa Ulmi Wehm.

Berkeley's type collection (Kew Herb., ex Berkeley Herb. No. 3866) of Sphaeria sudans is in reality closely related to the genus Melanconis. It differs from most species of Melanconis in the possession of larger spores which show a gelatinous envelope at first, in the slight development of the ectostroma, and in the conidial stage. These are all differences of degree but seem sufficient for subgeneric separation at least.

Massariovalsa sudans occurs on a number of different hosts. Berkeley's type on Acer from South Carolina and a later collection on *Ulmus* from Pennsylvania are the same as all other collections which have been examined. Ellis reports this species on Acer, Carya, and Quercus. The writer has collected it on Acer, Ulmus, and Nyssa and has received mate-

<sup>1</sup> Received for publication October 7, 1939. Papers from the Department of Botany of the University of Michigan No. 709.

rial from J. H. Miller on Acer, Nyssa, and Carya from Georgia.

Material on all these hosts is very similar (fig. 1). The perithecia are formed in small circinate groups in the upper bark cortex, have thick parenchymatic walls and cause a slight pustulate swelling of the periderm. There is very little ectostroma formed, and this is practically obliterated by the convergent emergent ostioles. The resulting appearance on the surface is a very minute black disc, consisting of the more or less fused ostioles erumpent through the center of a flat swelling, often of loosened periderm. The ascospores (fig. 6) vary in size from  $26-52 \times$ 13-18.5  $\mu$ , but there is no definite correlation between spore size and host. The spores from Acer tend to run somewhat longer than those on Ulmus and Carya, but this may be due to the larger number of collections examined on Acer. On Acer, the perithecial stage is commonly accompanied by the conidial stage. No conidial pustules have been seen on the other hosts, except those developed in culture. On Carya, the pustules are firmer, more definite, irregularly scattered, and the ectostroma is more easily seen. These differences are probably due to the character of the hickory bark, but when first seen it was taken for a Melanconis. Cultural studies have proven it to be the same as Massariovalsa sudans. The placement of this genus in the Massariaceae is undoubtedly due to the slight ectostromatic development of the disc and the gelatinous envelope found about the spores. This latter character is found in some species of *Melanconis* also, and cultural studies have shown that a definite ectostroma is formed. The paraphyses found in the young perithecium, furthermore, are broad, band-like, guttulate, and evanescent, as in *Melanconis*. The conidial stage is a modification of the Melanconium type with conidia formed within an enclosed chamber, instead of open cavities. The relationship is obviously with *Melanconis*.

In order to determine whether or not the occurrences of *M. sudans* on the various hosts represent separate species, single ascospore isolations were

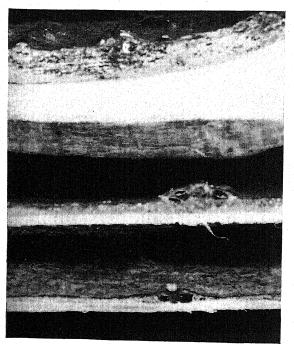


Fig. 1. Radial sections of stromata of Massariovalsa sudans (B. & C.) Sacc. on twigs of Acer saccharinum (above), Carya glabra (center) and Nyssa sylvatica (below).

made from three collections kindly sent the writer by J. H. Miller from Athens, Georgia. The hosts and dates of collection of these were as follows: Carya glabra, March 12, 1938; Acer rubrum, December 8, 1938; and Nyssa sylvatica, June 4, 1938. Certain cultural differences were observed between these host strains, but in general the results were similar. A general consideration of the strain from Carya will serve for all three strains. The varietal differences will be considered later.

Ascospores from Carya glabra were sprayed onto nutrient agar on March 26, 1938. Germinating spores were found within 24-48 hours. Only a small percentage of the spores germinated, and most of the germinating spores were cracked at the septum, with the two cells of the spore partially separated or entirely free from one another (fig. 7). Two to four, stout, gnarled, branching germ tubes,  $2.5-5~\mu$  in diameter, were put out from each spore, either from

the central ruptured septum or from the ends of the spore.

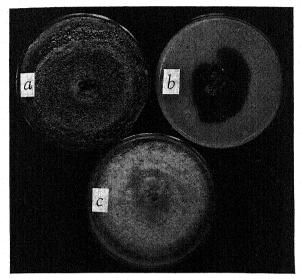
Growth was extremely slow on nutrient agar, colonies being only three to four centimeters in diameter after six months. Growth on oatmeal agar was somewhat more rapid. The surface of the agar is heavily blackened, and there is a superficial whitish to greenish-gray cottony mycelial growth. A few hemispheric stromata, of a similar greenish tint, were formed on oat agar after some six weeks. These split open, exposing a slimy inner surface. These stromata consisted of a mass of pale greenish to hyaline pseudo-parenchyma. In various internal areas, these cells put out short conidiophores which bore conidia at their apex. These conidia (fig. 8) were broadly ovoid, slightly flattened at the base where attached, hyaline at first but soon becoming olive brown and coarsely granular, finally wiith a thickened wall and homogeneous content and 26-33.5 imes 19.5-30  $\mu$ .

In the first twig inoculations the twigs dried out before fruiting occurred, because of the slow growth of the fungus. Autoclaved twigs of Carya sp. inoculated on November 2, 1938, showed numerous minute elevations of the periderm by December 29, and these later gave rise to erumpent, spheric, grayish stromata which finally showed the splitting off of a cap-like apical portion, similar to that found in agar cultures. The conidia were exuded in a black slimy mass and were identical with those found on agar.

Sections through these areas show that the pustules arose as rather widespread but interrupted areas of ectostroma on the bark surface. These ectostromata (fig. 4) are at first composed of loose, erect, hyaline, somewhat interwoven hyphae,  $2-3~\mu$  in diameter, causing a separation of the periderm from the bark cortex. These hyphae soon show a definite tendency to form a pseudo-parenchyma by the swelling and septation of the strands and the rounding up of the individual cells. This change occurs first at the base and in the central portions of these areas. As a result of subsequent increase in cell size, these central areas swell rapidly to an irregular spheric form and cause the pustulate ruptures of the periderm, and eventually the erumpent stromata.

The cells of this stroma are at first hyaline, filled with protoplasm and capable of continued growth. Upon exposure, the outer cells become thick-walled, olive-brown in color, and limit the expansion of the stroma. The inner cells remain hyaline and active, and at one or several points conidial formation is initiated. In these areas, the smaller more active cells may grow out directly as conidiophores, but the larger parenchymatic cells "sprout" by the rupture of the thin hyaline wall and the outgrowth of the naked protoplast as a conidiophore (fig. 9). The conidiophores are short, stout, 7-18  $\times$  2-4  $\mu$ , and often tapered to a point at the apex. The ruptured wall of the mother cell often appears as a sheath at the base. The conidiophores swell at the apex to form the ovoid conidium which is hyaline until it reaches nearly full size, when the wall becomes

thickened and a dark olive-brown. The conidia are  $21.5-27 \times 17.5-22~\mu$ . The formation of conidia continues centrifugally until one or a few large cavities result, limited by the outer wall of inert cells. The



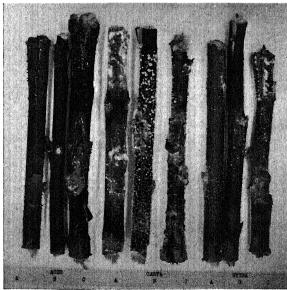


Fig. 2 (above). Colonies on oatmeal agar showing type of growth produced by strains of *Massariovalsa sudans* (B. & C.) Sacc. from (a) *Acer rubrum*, (b) *Carya glabra* and (c) *Nyssa sylvatica*.

Fig. 3 (below). Twig cultures of strains of Massariovalsa sudans (B. & C.) Sacc. from Acer, Carya and Nyssa, as indicated, on twigs of (A) Acer saccharum, (B) Carya ovata, and (C) Ulmus americana.

pressure resulting from spore production causes a rupture of the stroma, which usually takes place by the throwing back of a lid-like slice of the upper portion of the stroma.

Variations from the above life history are indicated in the following comparison of the three host

strains. Inoculations of the three strains were made in triplicate on oatmeal agar, and two series were carried for three weeks each. Certain definite differences appeared and were constant for each strain. These are described below and illustrated in figure 2.

On Carya: Colonies 2-4 cm. in diameter (after 3 weeks). Young colony with fine superficial tomentum which is grayish when viewed at a wide angle to the light. Soon forming a heavy dark green-black growth in the agar, giving the entire colony this color. Later a fine cottony growth of grayish mycelium may occur on the blackened stromatic areas. No pycnidia after three weeks.

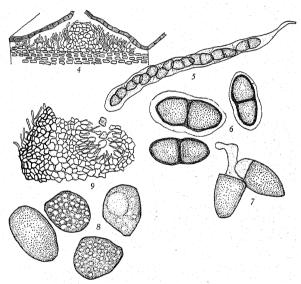


Fig. 4-9. Massariovalsa sudans (B. & C.) Sacc.—Fig. 4. Vertical section of young ectostroma, showing development of central plug of pseudoparenchyma.—Fig. 5. Ascus.—Fig. 6. Ascospores.—Fig. 7. Germinating ascospores.—Fig. 8. Conidia as formed in culture.—Fig. 9. Section of conidial locule and wall as formed in culture.

On Acer: Colonies 3-7 cm. in diameter. General color of the colony yellow-brown. Growth in concentric fan-like waves forming an outer tufted, superficial, yellow-gray mycelium behind which there is a more appressed superficial growth of darker yellow-brown hyphae arranged in a radiating system of very fine, loosely floccose, mycelial strands. Numerous grayish, superficial, spheric stromata, 0.5-1.0 mm. in diameter, with amber colored droplets present.

On Nyssa: Colonies 7-8.5 cm. in diameter. Surface growth of flocculent white mycelium, continuous or zonate in some colonies, 0.1-0.2 mm. thick. Agar with a characteristic tan to red-brown discoloration. Numerous minute, greenish-black stromata within the agar, which later proved to be perithecia.

On March 26, 1939, each of the strains from Acer, Carya, and Nyssa were inoculated onto three twigs each of Acer, Carya, and Ulmus (Nyssa twigs not being available). Figure 3 shows one set from this

series after eight weeks' growth. The growth of any one strain on the three hosts was very similar. There was somewhat greater superficial mycelial growth in the case of the Carya strain, but this may have varied with air moisture. The lack of growth of the Acer strain on Carya, for instance, was due to the drying out of the twig. The more important differences are cited below.

The Nyssa strain failed to produce any conidial stage in any of the many cultures carried, either on agar or on twigs. On the other hand, perithecia were produced in all these cultures but in none of the cultures from the other hosts. The perithecia formed were small and abnormally developed, but typical mature two-celled, brown ascospores were found in many of them.

The pycnidia formed by the strain from Acer did not open by the cap-like splitting of the apex, as is common in the Carya strain. The conidia of the Acer strain were exuded through a pore-like opening as fine coiled tendrils, rather than shapeless spore masses. The conidia of the Acer strain on twigs of Acer  $(23-32\times19.5-23\,\mu)$  and Ulmus  $(26.5-32\times18-21.5\,\mu)$  were slightly narrower and more ovoid than those of the Carya strain  $(26.5-33.5\times21-25\,\mu$  on Acer and  $26.5-39\times23-30\,\mu$  on Ulmus), but on twigs of Carya, both strains produced similar conidia  $(26.5-33.5\times21-25\,\mu$  for Acer strain and  $25-33.5\times21-26.5\,\mu$  for Carya strain).

These differences between the strains from different host genera are largely ones of physiological behavior, rather than of morphology and are, therefore, considered to be of varietal rather than of specific rank.

The conidial stage, obtained both in nature and in culture, is a Melanconiopsis. Ellis (1900) in describing this genus says "doubtless a stylosporous stage of a Melanconis or a Massariovalsa," and his type collection (N. Y. Bot. Gard., Ellis Coll., Kans., 1900, Bartholomew 2519) of Melanconiopsis inqui-

nans E. & E. on Acer dasycarpum is identical with the conidial stage obtained from the strain on Acer, showing the similar, somewhat narrower, conidia,  $25-35 \times 14-18 \mu$ .

This form genus can be considered as a modified Melanconium with enclosed locules. The differences between Massariovalsa and Melanconis might be considered of generic rank, but inasmuch as the genus Melanconis, as now circumscribed, has species with various affinities and variable conidial stages, the writer would prefer to place Massariovalsa as a sub-genus of Melanconis. These arrangements within the genus Melanconis will be considered in a forthcoming paper.

#### SUMMARY

Cultural studies of three strains of the one good species of Massariovalsa, M. sudans (B. & C.) Sacc., from Acer, Carya, and Nyssa, indicate certain differences considered to be of varietal rank.

The strains from Acer and Carya produced a conidial stage, identical with Melanconiopsis inquinans E. & E. The strain from Nyssa produced no conidia but numerous perithecia in all cultures.

The genus Massariovalsa is considered as a subgenus of Melanconis.

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# HYDROGEN-ION CONCENTRATION OF LEAF JUICE IN RELATION TO ENVIRONMENT AND PLANT SPECIES <sup>1</sup>

# Annie M. Hurd-Karrer

THE DATA reported in the present paper show the extent to which the acidity of the wheat plant is subject to change by the environment. Hundreds of pH determinations for plants in all stages of development, grown under different environmental conditions over a period of years, are summarized in the form of distribution curves that permit determination of the most typical value and the range of variation. Some of the data are tabulated to illustrate specific effects of growth factors.

Comparative acidities of other kinds of plants are shown by a compilation of pH values reported by other investigators.

<sup>1</sup> Received for publication October 23, 1939. Numbers in parentheses refer to Literature Cited at end of this article. The significance of acidity measurements on the expressed juice of plants is enhanced by Chibnall and Grover's conclusion (7) that since the buffering power of the vacuole fluid is greater than that of the cytoplasm the hydrogen-ion concentration of the expressed juice is a close approximation to that of the vacuole fluid in the living cell.

METHODS AND MATERIAL.—The plant material was macerated by grinding in a food grinder, or in a Nixtamal mill, and the juice expressed by squeezing it out by hand through muslin or cheesecloth. It was found that preliminary freezing of the plants had little effect on the hydrogen-ion concentration of the expressed juice. The use of high pressure has been advocated; but the writer found that values for

juice expressed by hand from a thoroughly macerated pulp did not differ from those of juice expressed by pressure up to 2,000 pounds per square inch (table 1). Small differences in concentration can have no appreciable effect on the pH value of a juice as highly buffered as that of wheat (20). Hence the simpler method, which facilitated the work without seeming to introduce detectable errors, was used throughout.

Table 1. pH values of four successive expressions, with increasing pressure, of juice from eight different lots of wheat leaves.

Pressure			L	eaf sa	mple r	10.		
(lbs. per sq. in.)	1	2	3	4	5	6	7	8
a	5.65	5.77	5.74	5.82	5.88	5.94	6.03	6.18
500	5.67	5.77	5.75	5.82	5.91	5.93	6.03	6.2
1,000	5.66	5.77	5.76	5.82	5.94	5.94	6.03	6.20
2,000	5.67	5.76	5.77	5.82	5.94	5.93	6.01	6.20

<sup>&</sup>lt;sup>a</sup> Juice squeezed from ground tissues by hand through cheesecloth.

The hydrogen-ion measurements were made electrometrically with the hydrogen electrode and saturated calomel cell. Most of the measurements were made at a temperature of 25°C. When there was any departure from this temperature, the proper corrections were made in the calculations. The equipment and details of the method have been described previously (20). Frequent checking with M/20 potassium acid phthalate showed the system to be accurate to within  $\pm .01$  pH.

Wheat plants were grown in a fertile soil (composted Keyport clay loam) having a reaction near pH 7.0. Unless otherwise specified the measurements are for the juice of green leaves, dead or yellowish ones having been discarded. To avoid complications from environmental conditions other than the one under consideration, the plants of a given experiment were always cut at as near the same time as possible, usually in the morning.

EXPERIMENTATION.—Effects of temperature.—
The data recorded in table 2² indicate that the acidity of wheat varies directly with the temperature at which the plants are grown—i.e., the warmer the greenhouse the lower were the pH values obtained for the juice. All three varieties included in the experiment grew best at the lowest temperature, but they differed with respect to severity of injury sustained at the higher temperatures. Turkey, a winter variety, grew poorly and remained vegetative at temperatures above 20°C., while Hard Federation, a spring variety, grew fairly well at temperatures as high as 20° to 25°, but poorly at 25° to 30°. Table 2 shows that Turkey became much more acid at temperatures above 20° than did Hard Federation. Harvest Queen was intermediate between the

<sup>2</sup> This table is a rearrangement of data published previously (25) plus some additional measurements for the same lots of plants after the period of temperature control (i.e., after 18 weeks).

with respect to both temperature requirement and acidity. Therefore it is concluded that the degree of acidification produced by high temperature is determined by the susceptibility of the variety to high-temperature injury. No varietal differences were found in any experiment where temperatures were low enough for equally vigorous growth and development of all the plants.

Table 2. pH values of wheat juice in relation to temperature. (Temperatures controlled up to and including the age of 18 weeks.)

	Temperature					
Variety and age	12–18°C.	20–25°C.	25–30°C.			
Hard Federation (spring type)						
5 weeks	. 6.25	6.02	6.04			
8 weeks	. 6.08	6.01	5.90			
9 weeks	. 6.16	6.10	6.04			
12 weeks	6.12	6.04	5.96			
15 weeks		6.10	5.79			
18 weeks		6.07a	5.74b			
Harvest Queen (intermediate type)						
5 weeks	. 6.15	6.04	5.94			
8 weeks		5.98	5.92			
9 weeks		6.02	5.84			
12 weeks	. 6.09	6.04	5.80			
15 weeks		5.90	5.64			
18 weeks		5.85	5.65			
20 weeks	. 5.90 <sup>a</sup>	5.83				
24 weeks	. 5.89	5.62°	$5.62^{d}$			
Turkey (winter type)						
5 weeks	. 6.16	6.04	5.91			
8 weeks		6.09	5.84			
9 weeks		5.90	5.83			
12 weeks		5.95	5.79			
15 weeks		5.85	5.65			
18 weeks		5.65	5.63			
20 weeks		5.52	5.65			
24 weeks		5.39 <sup>d</sup>	5.49 <sup>d</sup>			

- a Flowering; normal development.
- <sup>b</sup> Sterile heads.
- c Flowering; retarded development.
- d No heads; plants severely injured.

Effects of day length.—Table 3 gives data obtained with Turkey, a winter wheat, showing that the pH value varied inversely with length of the daily period of light. Decreasing the light period from the natural day to 8 hours, by methods described previously (27), produced vegetative, unfruitful plants with consistently higher pH values than those of adjacent control plants with a normal day of 9 to 15 hours; and lengthening the light period to 17 hours by means of artificial illumination produced stunted, prematurely ripening plants with pH values generally lower than those of the controls.

In some later experiments with a spring variety (cf. table 6) shortening the light period to 8 hours had no effect on the measurements, although length-

Table 3. pH values of wheat (Turkey) in relation to day length, soil reaction, and age.

Age of plants in		Day le	ngth	
weeks	8 hrs.	9½ to 15 hrs.	17 1	noursa
		(natural day)	$\mathbf{A}$	В
Grown at 10 to 14°(	) <b>.</b>			
6 <sup>b</sup>	6.07	5.98	6.00	5.95
7		6.04	5.96	5.96
8		6.06	5.95	5.95
8b	6.03	5.94	5.92	5.92
11	6.09	6.00	5.96	5.96
12 <sup>b</sup>	6.28	6.08	6.03	5.94
14	6.09	6.03	5.77	5.70
14 <sup>b</sup>	. 6.09	5.86	5.68	5.67
Grown at 20 to 24°C	<b>!.</b>			
3		6.01	5.92	5.90
3 <sup>b</sup>	. 6.05	5.99	5.91	5.90
4	. 5.99	5.88	5.86	5.82
4 <sup>b</sup>	6.10	6.05	5.96	5.94
6	. 5.91	5.88	5.81	5.88
$6^{\mathrm{b}}\dots\dots$	. 5.99	5.89	5.85	5.84
8	6.00	5.92	5.79	5.76
$8^{\mathrm{b}}$	6.07	5.94	5.90	5.90
9 <sup>b</sup>	. 5.99	5.90	5.82	5.83
11	6.10	5.98	5.90	5.84
11 <sup>b</sup>	. 6.01	5.92	5.75	5.75
15 <sup>b</sup>	. 5.99	5.65	5.80	5.67
16	. 5.89	5.85	5.65	5.67
$16^{\mathrm{b}}\dots$	. 5.81	5.63	5.73	5.72
27	. 5.66	5.52	5.41	5.41
27 <sup>b</sup>	. 5.59	5.32	5.40	5.31

<sup>&</sup>lt;sup>a</sup> Supplementary light was (A) 15 to 50 foot-candles and (B) 50 to 100 foot-candles.

<sup>b</sup> Plants grown in limed soil (pH 8.1). All others grown in unlimed soil (pH 7.1).

ening it to 17 hours increased the acidity in two out of three experiments.

Effects of late sowing.—Wheat sown too late in the season for normal development is characterized by abnormally high acidity, especially spring-sown winter wheat whose heading is inhibited. Table 4 shows the increasing acidity of poorly developing plants of both a spring (Jenkin) and a winter (White Odessa) variety sown in a greenhouse at Arlington, Virginia, in March, compared with corresponding measurements on vigorous plants of January sowings. Tables 2 and 3 show that both high temperature and long days are factors in the late spring environment that tend to bring about this acid condition.

Newton et al. (42) give data that show such an effect of late sowing on the acidity of the winter variety, Kanred. Spring varieties sown at the same time maintained a normally low acidity, with pH values between 5.91 and 6.35, over a period during which the acidity of Kanred increased to pH 5.64. Kanred is described as having "behaved in the usual manner of a winter wheat seeded in the spring, failing to joint or progress toward maturity." In a later paper (41) Newton and Brown report abnormal

acidities increasing from pH 5.60 to 4.87 over a period of six weeks in young plants of very late (June 9) sowings of Kanred.

Effects of light intensity.—The considerable difference in intensity of the artificial light supplied from sunset to midnight to two different lots (A and B) of long-day wheat (table 3) had no effect on the pH value. Significantly, there was no detectable difference in the appearance nor rate of development of the plants.

Many plants, especially fleshy or succulent ones (29), show pronounced diurnal changes in acidity, but those of wheat are small. Series of measurements on wheat for the entire 24-hour period are given by Loehwing (33). They show that the pH value is at a minimum in the early morning and at a maximum in the evening. Differences between morning and afternoon cuttings similar to those of Loehwing have also been reported by the writer (20).

Effects of etiolation.—The acidity of juice from etiolated wheat seedlings grown for a week to 10 days in the dark (in unlighted germinators at temperatures near 20°C.) averaged a little higher than that for green seedlings. The difference is shown by the data of table 5 and by another group discussed later in connection with frequency distributions (fig. 1). However, differences were small, and the ranges overlapped. Moreover, the probability of variation in other factors of the germinator and greenhouse environments, such as temperature and humidity, preclude attributing the acidity differences to etiolation alone with any degree of certainty. More significant than their difference is their similarity, indicative of effective functioning of the buffer system before initiation of photosynthetic processes. There is evidence (26) that inorganic phosphates are the

Table 4. pH values for greenhouse-grown wheat in relation to planting date at Arlington, Virginia.

	White Odessa		Jenkin		
Age in weeks	Sown Jan. 13 Developed normally	Sown Mar. 10 Developed poorly	Sown Jan. 13 Developed normally	Sown Mar. 24 Developed poorly	
1	6.07	5.95	6.00	6.00	
2	6.02	6.00	6.22	5.93	
3	6.21	6.11	6.11	5.86	
4	6.19	5.68	6.18	5.64	
5	6.35	5.61	6.36		
6	6.23	5.55	6.38	5.53	

buffers largely responsible for regulating the hydrogen-ion concentration of such plants as wheat.

Effects of soil reaction.—In order to determine whether liming the soil affects the acidity of wheat, calcium hydroxide was added at a rate of four tons per acre to composted Keyport clay loam. The limed and unlimed soils were in the same greenhouse bench but separated by a buffer compartment containing dry soil. The pH values for 1:1 water extracts were

Table 5. pH values for etiolated and green wheat seedlings grown at about 20°C.

	Experi-		<b>3377. :</b> 1.						
Condition of seedlings	ment no.	Hussar	White Odessa	Ridit	Florence	Martin	Jones Fife	Hybrid 128	Jenkin
Etiolated, grown in									
germinators at 20°C.	1	5.91	6.03	6.03	6.03	5.96	5.93	5.93	5.84
	2	5.89	6.03	6.00	6.02	5.93	5.90	5.90	5.85
	3	5.89	6.01	6.02	6.02	5.93	5.87	5.88	5.86
	4	5.91	6.01	5.98	•••	5.96	5.85	5.85	5.89
Average	••	5.90	6.02	6.01	6.02	5.95	5.89	5.89	5.86
Green, grown in soil at									
approximately 20°C.	1	6.01	6.07	6.11	6.11	5.99	5.95	5.96	6.12
	2	6.03	6.04	6.10	6.12	5.96	5.98	5.89	6.06
	3	6.14	6.01	6.07	6.03	6.04	5.85	5.85	5.89
	4	•••	6.03	6.16	5.94	6.13	5.97	6.00	5.88
Average		6.06	6.04	6.11	6.05	6.03	5.94	5.93	5.99

6.95 to 7.28 for the unlimed soil and 8.01 to 8.18 for the limed soil.

The data of table 6 show that the acidity of the wheat juice was not decreased by the lime. In the experiment of table 3, also, there was no appreciable difference between values for plants from limed and unlimed soils. In both of these experiments, vigor of growth was not affected by the lime. In some earlier experiments (20) it had reduced the juice acidity by as much as 0.2 pH, interpreted as an indirect result of the favorable effect of lime on growth in the particular soil used.

Limits of variation and the range for normal plants.—The pH values for wheat reported in this and previous papers (20, 22, 23, 25), together with other unpublished data, are assembled in the frequency distribution curves of figure 1.

The largest group, that of curve no. 1, comprises all the data—i.e., pH values for 707 different samples of juice from green plants of all ages and en-

Table 6. pH values for young Hard Federation wheat plants grown in unlimed (pH 7) and in limed (pH 8) soil.

	Exper	iment 1	Exper	iment 2	Experiment 3		
Day length	Un- limed	Limed	Un- limed	Limed	Un- limed	Limed	
8 hours	6.08	6.09	5.98	5.94	5.97	6.02	
10 hours	6.03	6.10	5.93	5.95	5.98	5.99	
17 hours	6.08	6.08	5.87	5.90	5.88	5.95	
17 hours	6.07	6.11	5.87	5.87	5.85	5.93	

vironments. They range from pH 5.31 to 6.38, with 80 per cent of them falling between 5.81 and 6.20. The frequency distribution curve is not normal but unsymmetrical because of extension on the left, in the direction of values more acid than the mean. Obviously this form results from inclusion of a relatively small number of plants more acid than most of the material of the experiments. These more acid plants are (1) the older ones, in the maturation

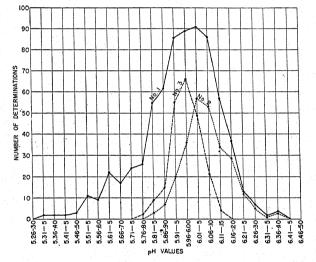


Fig. 1. Frequency distributions of pH values for three groups of wheat plants: No. 1, green plants irrespective of age or condition; No. 2, the same group after elimination of data for plants older than 12 weeks and for unhealthy plants; No. 3, week-old seedlings grown without light.

period, and (2) those in an abnormal or unhealthy condition from any cause, such as late spring sowing, unfavorably high temperatures, or artificially lengthened light periods.

When the data for the older and the unhealthy plants are omitted from curve no. 1 (specifically, values for plants beyond the tillering stage, or older than 12 weeks, and for plots sown later than March 1, or in greenhouses where temperatures were above 20°C. or where light periods were other than the normal day length) frequency curve no. 2 (of figure 1) is obtained. The 260 values range from 5.85 to 6.38 about a mean at pH 6.07. Their distribution conforms so closely to a normal curve calculated from the data (fig. 2) as to permit the inference that the deviations from the mean are only those to be expected as random variations of an essentially homo-

geneous population.<sup>3</sup> Such homogeneity was unexpected, because no attempt had been made to produce identical environments for the several different years' experiments that supplied the data. Evidently the variables in the environment that remained after the eliminations noted had little effect on the plant

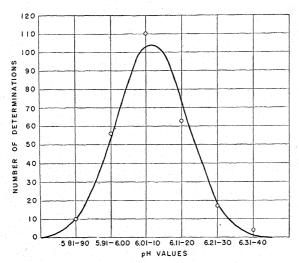


Fig. 2. Normal distribution curve calculated from pH values of juice from healthy vigorous wheat plants not more than 12 weeks old. (Calculations made and curve drawn by O. E. Rauchschwalbe of the Division of Cereal Crops and Diseases, Bureau of Plant Industry.)

acidity. It can be concluded that the range pH 5.9 to 6.2, within which 88 per cent of the values fall, represents the most usual acidity of wheat in the vegetative stage.

During the maturation period, beginning usually some time before flowering, there is a progressive increase in acidity (22, 24), sometimes to pH values as low as 5.3 (tables 2 and 3). Other data showing this trend have been published by Newton and Brown (41) for wheat, Haas (14) for buckwheat, Miyake and Adachi (37) for oats and flax, May (36) for tomato, Górski and Dabrowska (13) for legumes, and Garner et al. (12) for tobacco.

It is of interest to note the narrow range of variation in pH values obtained for etiolated wheat seedlings of 23 different varieties grown for a week to 10 days in unlighted germinators with temperature controlled automatically at about 20°C. The range for the 221 values, whose distribution is shown in curve no. 3 of figure 1, was from 5.80 to 6.14, with 86 per cent between 5.91 and 6.10, and conforming, as shown by the chi-square test, to a normal distribution about a mean at pH 5.97.3 The somewhat narrower range of values as compared with the corresponding range for the green plants presumably reflects the greater constancy of the environment in the germinators as compared with that in the greenhouse.

<sup>3</sup> It is recognized that such interpretations of statistical treatments of pH values—i.e., of negative logarithms—may be open to question.

PH VALUES REPORTED FOR OTHER CROP PLANTS.—
Hydrogen-ion determinations reported in the literature for other crop plants are summarized in table 7. The compilation has been limited with few exceptions to electrometric measurements on the juice of leaves, or occasionally of whole tops, of plants subjected to normal growth conditions. Sometimes, however, the exact tissues used were not specified by the investigator. Sometimes the range of values given is inclusive for varying conditions produced for the particular investigation, such as different hours of sampling and different soil conditions. It is therefore impossible to assure strict comparability of the figures, especially since differences in age and vigor will also have affected them.

The data of table 7 show that the juice of many crop plants is generally, like that of wheat, near pH 6.0. Distinctly more acid than this, but above pH 5.0, are buckwheat, corn, cowpea, the sorghums, apparently Sudan grass and sugar cane, and apparently some of the lupines and crucifers. Most acid of all are rhubarb and apparently onion. The lowest acidities, occasionally above pH 7.0, have been reported for hemp, pumpkin, squash, sunflower, sweet clover, and field pea.

It is interesting to note that the Gramineae in the list, comprising two distinct types morphologically, comprise two corresponding groups from the standpoint of acidity. These are (1) the small grains—barley, oats, rye, and wheat, with pH values near 6.0 on the average; and (2) a more acid group comprising corn, sorghums, Sudan grass, and sugar cane, with pH values generally near 5.5.

Measurements for some highly acid tissues are summarized in table 8 for comparison with the crop plants. The extremely high concentrations of acid indicated by some of the figures are undoubtedly localized in special cells or vacuoles, hence not in contact with the protoplasm, nor with the enzymes that are inactivated by such acidities (28).

Discussion.—Evidently in plant as in animal tissues (31, 38, 46) various types of injury are associated with acidification. Several investigators (15, 16, 20, 45, 47, 49, 51, 52) have found that diseased plants are more acid than corresponding healthy ones. That the pH values of wheat and corn vary with vigor has been reported previously (20, 21, 22) and a similar correlation appears in the work of Newton et al. (41, 42) where Kanred wheat, unable to develop normally because of late sowing, became abnormally acid. Also Loehwing (33) reports a lower acidity for vigorous wheat in a fertile (unlimed) soil than for less vigorous plants in a nonfertile soil. Haas (14) found that the second year's growth of alfalfa was not as acid as the less vigorous first year's growth; Dustman (10) that in some tomato seedlings grown with different quantities of calcium in the nutrient solution the acidity of the sap decreased with increasing vigor of growth; Eckerson (11) that phosphate-deficient tomato plants are abnormally acid, being essentially nitrogen-starved even though grown with an adequate

Table 7. Compilation of pH values reported by other investigators for juice of leaves of crop plants.<sup>a</sup> (References are to pages where the cited values occur.)

Crop	pН	Investigator	Reference
Alfalfa (Medicago sativa)	5.67 5.71–5.94 5.99–6.19 5.84–6.22 6.16–6.31 5.57–6.46	Chibnall and Grover Clevenger Haas A. L. Pitman Shywan Truog and Meacham	Biochem. Jour. 20: 112 Soil Sci. 8: 233 Soil Sci. 9: 352 Unpublished Pflanzenbau 6: 171, 173 Soil Sci. 7: 470
Asparagus (Asparagus officinalis)	6.09 5.69-6.09	Fife and Frampton Ingalls and Shive	Jour. Biol. Chem. 109: 647 Plant Physiol. 6: 117
Barley (Hordeum vulgare)	5.7b -6.0b 5.62-5.72 5.68-6.20 6.0 -6.2 5.93-6.26 5.9 -6.1	Arrhenius Haas Hoagland Keyssner A. L. Pitman Theron	Ark. Bot. 18 (1): 33 Soil Sci. 9: 355 Bot. Gaz. 68: 299 Planta 12: 579 Unpublished Univ. Calif. Publ. Agric. Sci. 4: 424
Bean (Phaseolus spp.)	5.66-6.21 5.92-6.12 5.92-6.10 5.65-5.97 5.55-6.00 6.0 -6.1	Acharya and Sastri Chibnall and Grover Gustafson Haas Kappen and Zapfe Keyssner	Jour. Ind. Inst. Sci. 14A: 4 Biochem. Jour. 20: 112 Amer. Jour. Bot. 11: 2 Soil Sci. 9: 355 LandwVersuchssta. 93: 140 Planta 12: 579
Bean (Vicia faba)	5.67-5.84 5.76-6.47 5.55-5.95 6.05-6.21	Chibnall and Grover Gorski and Dabrowska Jancke Phillips	Biochem. Jour. 20: 112 [Polish Agric. and For. Ann.] 14: 450 Phytopath. Zeitschr. 2: 195 Bot. Gaz. 69: 174
Beet (Beta vulgaris)	5.68-6.33 5.92 5.95-6.61 5.96-6.29 5.43-6.16	J. S. Caldwell Dittrich Fife and Frampton Mumford Stoklasa	Unpublished Planta 12: 114 Jour. Biol. Chem. 109: 646-652 Ann. Appl. Biol. 17: 30 Biochem. Zeitschr. 176: 49
Buckwheat (Fagopyrum esculentum)	5.28-5.93 4.70 <sup>h</sup> -5.43 <sup>h</sup> 5.01-5.03 5.27-5.62 5.48-5.97 4.82 <sup>c</sup> 4.90-5.38 5.7 -6.0 5.52-5.89 4.4	Clevenger Clevenger Domontovitsch Dunne Haas Haas Ingalls and Shive Keyssner Neller Olsen	Soil Sci. 8: 237 Soil Sci. 8: 237 Jour. landw. Wiss. (Moscow) 2: 707 Hilgardia 7: 226 Soil Sci. 9: 352 Soil Sci. 9: 352 Plant Physiol. 6: 109 Planta 12: 579 Jour. Agric. Res. 51: 292 Compt. Rend. Trav. lab. Carlsberg 16: 17
Cabbage (Brassica oleracea capitata)	4.02-4.05 5.5 5.60-5.68 6.0 <sup>d</sup> -6.4 <sup>d</sup> 6.31-6.34 5.60-5.84 <sup>e</sup> 5.90 5.89-5.94	Truog and Meacham Bigelow and Cathcart Chibnall and Grover Chibnall and Grover Domontovitsch Harvey McClendon and Sharp A. L. Pitman	Soil Sci. 7: 470  Nat. Canners' Assoc. Bull. 17-L: 32  Biochem. Jour. 20: 112  Biochem. Jour. 20: 112  Jour. landw. Wiss. (Moscow) 2: 707  Jour. Agric. Res. 15: 101  Jour. Biol. Chem. 38: 533  Unpublished
Carrot (Daucus carota)	5.67-5.90 5.67	J. S. Caldwell Shywan	Unpublished Pflanzenbau 6: 173
Clover, red (Trifolium pratense)	5.94-6.19 5.94-6.08 5.69-5.90	Haas Shywan Truog and Meacham	Soil Sci. 9: 355 Pflanzenbau 6: 171 Soil Sci. 7: 470
Clover, white (T. repens)	5.85–6.36 6.18–6.37	Ingalls and Shive Shywan	Plant Physiol. 6: 109, 117 Pflanzenbau 6: 171, 173
Clover, alsike (T. hybridum)	6.12-6.27 5.28-6.19	Shywan Haas	Pflanzenbau 6: 171, 173 Soil Sci. 9: 355

Table 7 (continued).

Crop	pН	Investigator	Reference
Corn (Zea mays)	5.30-5.50	Barton	Trans. Kansas Acad. Sci. 36: 74
	5.31-5.49	Bauer and Haas	Soil Sci. 13: 466
	5.11-5.21	Bryan	Soil Sci. 13: 281
	5.66	Chibnall and Grover	Biochem. Jour. 20: 112
	5.74 - 5.76	Domontovitsch	Jour. landw. Wiss. (Moscow) 2: 707
	5.00 - 5.58	Gustafson	Amer. Jour. Bot. 11: 2
	5.19 - 5.48	Haas	Soil Sci. 9: 355
	5.34-5.68	Hurd	Jour. Agric. Research 25: 459, 462
	5.3 - 5.9	Keyssner	Planta 12: 580
	5.34-5.73	Neller	Jour. Agric. Res. 51: 291
	5.6 - 5.7	Theron	Univ. California Publ. Agric. Sci.
			4: 424
	5.21-5.31	Truog and Meacham	Soil Sci. 7: 470
	5.31 - 5.74	Wadleigh and Shive	Soil Sci. 47: 276, 280
Cotton (Gossypium)	5.25-5.46	Harris, et al.	Science 61: 65. Also, Jour. Agric. Res 27: 297, 307
	5.6	Humfeld	Unpublished
	5.89	Mason and Maskell	Annals Bot. 42: 587
Cowpea (Vigna sinensis)	5.33-5.53	Bryan	Soil Sci. 13: 281
Company ( regime outcomes)	5.28-5.81	Clevenger	Soil Sci. 8: 234
		Clevenger	Soil Sci. 8: 234
	5.57-5.68	H. L. Hyland	Unpublished
	5.19-5.79	A. L. Pitman	Unpublished
Flow (Linum scatteringimum)	5.37-5.76	Miyake and Adachi	Jour. Biochem. (Tokyo) 4:320
Flax (Linum usitatissimum)	5.80-5.89	A. L. Pitman	Unpublished
Grasses	5.92-6.11	Haas	Soil Sci. 9: 357
	5.27 - 6.02	Martin and Brown	Canadian Jour. Res. 3: 400
	5.63 - 6.34	Shywan	Pflanzenbau 6: 172, 173
Kale (Brassica oleracea acephala)	5.12-5.70	J. S. Caldwell	Unpublished
Hemp (Cannabis sativa)	7.12	Shywan	Pflanzenbau 6: 172
Lupine (Lupinus albus)		Gorski and Dabrowska	[Polish Agric. and For. Ann.] 14: 450
			451
	5.78-6.03	Hempel	Compt. Rend. Trav. lab. Carlsberg 13
			116
	5.94	Shywan	Pflanzenbau 6: 172
Lupine (L. angustifolius)	5.80 - 6.47	Gorski and Dabrowska	[Polish Agric. and For. Ann.] 14: 45
Lupine (L. hirsutus)	5.45-5.65	Truog and Meacham	Soil Sci. 7: 470
Lupine (L. luteus)	3.87-5.98	Gorski and Dabrowska	[Polish Agric. and For. Ann.] 14: 451
			452
	5.31-5.63 5.22-5.44	Haas	Soil Sci. 9: 355
		Kappen and Zapfe	LandwVersuchssta. 93: 140
Lupine (L. perennis)	5.35-5.37	Shywan	Soil Sci. 6: 171, 173
Mustard (Sinapis alba)	5.48-5.78	Haas	Soil Sci. 9: 352
	5.5	Wagner	Centralbl. Bakt. 44 (Abt. 2): 711
Oats (Avena sativa)	6.4 - 6.6	Arland	Bot. Arch. 7: 98
	5.99-6.18	Bonner	Protoplasma 21: 414, 415
	5.72-5.97	Clevenger	Soil Sci. 8: 237
	5.52b-6.10b	Clevenger	Soil Sci. 8: 237
	6.13 - 6.14	Domontovitsch	Jour. landw. Wiss. (Moscow) 2:707
	5.65-5.67	Haas	Soil Sci. 9: 355
	5.9 - 6.5	Keyssner	Planta 12: 579
	5.57-6.49	Miyake and Adachi	Jour. Biochem. (Tokyo) 4: 318
Onion (Allium cepa)	4.33-4.50	Smith and Quirk	Phytopath. 16: 504, 505
	4.8 -5.2	Walker	Jour. Agric. Res. 24: 1035
	4.8 -5.0	Yamaha and Ishii	Protoplasma 19: 208
Pea, field (Pisum arvense)	6.47-7.50	Gorski and Dabrowska	[Polish Agric. and For. Ann.] 14: 45
			458
	6.53 - 6.80	Haas	Soil Sci. 9: 352
	5.93 - 6.05	A. L. Pitman	Unpublished
	6.15	Shywan	Pflanzenbau 6: 172

TABLE 7 (continued).

Crop	pН	Investigator	Reference
Pea, garden (P. sativum)	6.0 -6.4	Keyssner	Planta 12: 579
	6.57	Shywan	Pflanzenbau 6: 172
	5.7 -5.9	Theron	Univ. California Publ. Agric. Sci. 4: 424
	6.2	Tropova	[Jour. Agric. Res. No. Caucasus] 13: 12
Potato (Solanum tuberosum)	5.5	Ingold	Protoplasma 9: 445
	5.70-5.72	Boas	Zeitschr. Pflanzenkrankh. 29: 173
	5.8	Wagner	Centralbl. Bakt. 44 (Abt. 2): 713
	6.19 - 6.58	Rogers and Shive	Plant Physiol. 7: 235
Pumpkin (Cucurbita pepo)	6.80 - 7.50	Gustafson	Amer. Jour. Bot. 11: 4
	5.8 -6.1	Keyssner	Planta 12: 579
	6.73-7.15	A. L. Pitman	Unpublished
Radish (Raphanus sativus)	5.30-5.92	Pearsall and Ewing	Annals Bot. 43: 29, 31
Rape (Brassica napus)	6.00 - 6.51	Neller	Jour. Agric. Res. 51: 297
	5.30-5.84	A. L. Pitman	Unpublished
	5.10-5.18	Truog and Meacham	Soil Sci. 7: 470
Rhubarb (Rheum rhaponticum)	4.00-4.62	Chibnall and Grover	Biochem. Jour. 20: 112
	3.36 3.1	Haas Patten and Mains	Soil Sci. 7: 490 Jour. Bact. 2: 221
	3.09	Rohde	Pflügers Arch. 168: 422
	3.4 -4.1	Vickery, et al.	Connecticut Agric. Exp. Sta. Bull. 424:
			40
Rice (Oryza sativa)	5.29-6.25	Miyake and Adachi	Jour. Biochem. (Tokyo) 5: 323, 324
	5.83-6.79	Hirayama	Forsch. Geb. Pflanzenkr. K. Univ. Kyoto 1: 23
Rye (Secale cereale)	6.03	Domontovitsch	Jour. landw. Wiss. (Moscow) 2: 707
aye (Socaro coroaro)	6.0 - 6.2	Keyssner	Planta 12: 575
	5.93	A. L. Pitman	Unpublished
	6.1	Tropova	[Jour. Agric. Res. No. Caucasus] 13:
Sorghum (Surghum vulgare):			12
Saccharine	5.3 -5.5	Keyssner	Planta 12: 580
	5.56 - 5.58	A. L. Pitman	Unpublished
	5.62 - 5.63	Shywan	Pflanzenbau 6: 172
Non-saccharine:		A T 701	TT1:.15
Feterita	5.53-5.55	A. L. Pitman A. L. Pitman	Unpublished Unpublished
Milo	5.45-5.50 4.98-5.87	Neller	Jour. Agric. Res. 51: 290-294
Shallu		Bauer and Haas	Soil Sci. 13: 462
Soybean (Soja max)	5.82-6.61 5.60-6.16	Bryan	Soil Sci. 13: 281
	6.25 - 6.53	Clevenger	Soil Sci. 8: 238
	5.95b-6.411		Soil Sci. 8: 238
	5.97-6.80		
		Allard	Jour. Agric. Res. 27: 135-138
	5.85	Haas	Soil Sci. 7: 489
	5.88-6.23	Ingalls and Shive	Plant Physiol. 6: 117
	6.0 -6.4	Keyssner	Planta 12: 579
	6.09-6.82	Loehwing	Plant Physiol. 9: 576 Plant Physiol. 7: 235
	5.83-6.10 6.17	Rogers and Shive Shywan	Pflanzenbau 6: 172
	5.82-6.4	Strowd	Soil Sci. 10: 351
	5.81-5.94		Soil Sci. 7: 470
Spingeh (Spingeig claracea)	6.50-6.57		Biochem. Jour. 20: 112
Spinach (Spinacia oleracea)	6.0 -6.4	Knott	Cornell Univ. Agric. Exp. Sta. Mem 106: 44
	5.83-6.16	Loehwing	Proc. Soc. Exp. Biol. and Med. 30: 1217
	5.84-6.24		Unpublished
Squash (Cucurbita maxima)	6.40-6.70		Amer. Jour. Bot. 11:2
Sudan grass (Sorghum vulgare suda-			en e
nense)	5.70	A. L. Pitman	Unpublished
	5.68	Shywan	Pflanzenbau 6: 172

Table 7 (continued).

Crop	pН	Investigator	Reference
Sugarcane (Saccharum officinarum)	5.29-5.72	Neller	Jour. Agric. Res. 51: 291, 294
Sunflower (Helianthus annuus)	6.40	Chibnall and Grover	Biochem. Jour. 20: 112
	6.01	Dittrich	Planta 12: 114
	7.05-7.25	Garner, Bacon and	
		Allard	Jour. Agric. Res. 27: 146
	6.30-6.90	Gustafson	Amer. Jour. Bot. 11: 4
	5.8 -6.4	Keyssner	Planta 12: 579
	6.45-7.09	Loehwing A. L. Pitman	Plant Physiol. 9: 571 Unpublished
	6.50-6.72 6.45	Shywan	Pflanzenbau 6: 172
	6.6 - 6.7	Tropova	[Jour. Agric. Res. No. Caucasus] 13:
			12
Swedes (Brassica rutabaga)	5.27-5.81	Pearsall and Ewing	Annals Bot. 43: 29, 31
Sweetclover (Melilotus)	6.53-7.38	Haas	Soil. Sci. 9: 359
	6.17 - 6.20	A. L. Pitman	Unpublished
	6.2 - 6.71	Shywan	Pflanzenbau 6: 171, 173
Sweet potato, vines (Ipomoea batatas)	5.29-5.35	J. S. Caldwell	Unpublished
Swiss chard (Beta vulgaris)	5.75-5.89	J. S. Caldwell	Unpublished
	6.08-6.12	A. L. Pitman	Unpublished
Timothy (Phleum pratense)	6.12-6.19	Haas	Soil Sci. 9: 352
	6.03-6.14	Shywan	Pflanzenbau 6: 172, 173
Tobacco (Nicotiana tabacum)	5.03-6.00	Böning and Böning-	
	- 00 - 00	Seubert	Biochem. Zeitschr. 247: 44-48
	5.22-5.36	Fife and Frampton	Jour. Biol. Chem. 109: 647
	5.26-5.95	Garner, et al.	Jour. Agric. Res. 23: 33, 34
	5.60-6.36	Garner, et al.	U. S. Dept. Agric. Tech. Bull. 414: 4
	5.25-5.50 5.58-5.83	Harvey Ingalls and Shive	Jour. Biol. Chem. 42: 399
	5.85-6.02	Thornton	Plant Physiol. 6: 117
	5.1 - 5.9	Wenusch and Molinari	Contrib. Boyce Thompson Inst. 5: 408 Zeitschr. Untersuch. Lebensm. 76: 24
Tomato (Lycopersicum esculentum)	5.78	Dittrich	Planta 12: 114
	5.35-6.21	Dustman	Bot. Gaz. 79: 248, 249
	5.52 - 6.70	Eckerson	Contrib. Boyce Thompson Inst. Pl.
			Res. 3: 204, 209
	5.58-6.15	Ingalls and Shive	Plant Physiol. 6: 117
	5.98-6.23	May	Ohio Jour. Sci. 29: 262, 263
	5.38°-5.46°		Ohio Jour. Sci. 29: 262, 263
	5.53-5.68	Thornton	Contrib. Boyce Thompson Inst. Pl.
	5.9	Wynd	Res. 5: 408 Ann. Missouri Bot. Gard. 21: 423
Turnip (Brassica rapa)	5.8	Dittrich	
	5.36-5.83	Pearsall and Ewing	Planta 12: 114
	5.89-5.93	A. L. Pitman	Annals Bot. 43: 29, 31 Unpublished
	5.7	Shywan	Pflanzenbau 6: 173
Vetch (Vicia spp.)	6.34-6.36ь	A. L. Pitman	
	6.45-6.47	Shywan	Unpublished Pflanzenbau 6: 172
Wheat (Triticum)	6.0 -6.4		
	6.31-6.73	Arrhenius Bystrikov	Zeitschr. Pflanzenkrankh. 34: 100, 10
	0.10	~ystikov	Bull. Appl. Bot. Gen. and Pl. Breed 25: 143
	5.87-5.93	Dunne	Hilgardia 7: 210
	5.77-6.33	Haas	Soil Sci. 9: 352
	5.85-6.38	Hurd-Karrer	Present paper (also 20, 22, 23, 25, 27
		Hurd-Karrer	Present paper
	5.89-6.19	Hursh	Jour. Agric. Res. 27: 402
	6.0 - 6.1 $5.7 - 6.73$	Keyssner Kwasnikov	Planta 12: 579
	5.47-6.67	Loehwing	Jour. landw. Wiss. (Moscow) 6: 23-3
	5.64-6.35	Newton, et al.	Plant Physiol. 5: 296, 297
			Canadian Jour. Res. 1: 15
	5.78 - 6.44	Newton and Martin	Canadian Jour. Res. 3: 398, 399

TABLE 7 (concluded).

	Crop		pН	Investig	ator	Reference		=
Wheat	(Triticum)—conc	luded	6.00-6.25	Stieglitz		Zeitschr. Pflanzenernähr. Di Bodenku. 43: 154	ing. u.	-

\*Lists of pH values for other kinds of plants and tissues are given by Bigelow and Cathcart (Nat. Canners' Assoc. Bull. 17-L); Arrhenius (Arkiv Bot. 18 (1): 31); Blagoveschenski (Bull. Univ. Asie Centr. Tachkent 14: 13); Bogen (Planta 28: 554); Chibnall and Grover (Biochem. Jour. 20: 112); Domontovitsch (Jour. landw. Wiss. [Moscow] 2: 706-711); Fife and Frampton (Jour. Biol. Chem. 109: 647); Reiss (Le pH Intérieure Cellulaire, pp. 94, 115); Shywan (Pflanzenbau 6: 171-173); Thornton (Contrib. Boyce Thompson Inst. 5: 406-408); and Youden and Dobroscky (Contrib. Boyce Thompson Inst. 3: 358, 359).

b Whole tops.

<sup>e</sup> Old or unhealthy plants.
<sup>d</sup> Etiolated (headed) cabbage.

<sup>e</sup> The second figure is for petioles.
<sup>f</sup> The names Sorghum saccharatum and Andropogon sorghum also used.

nitrate supply; and Nightingale et al. (43) that acidity is usually higher in calcium-deficient plants than in more vigorously growing plants with adequate calcium. Truog and Meacham (50) refer to the fact that with the development of hyperacidity there is slow growth and a weakened condition, "as is the case with plants of high lime requirement growing on acid soils." Hoagland and Martin (19) raise a question as to whether any considerable modification in the normal reaction occurs except when accompanied by definite injury to the plant. One then wonders whether abnormal acidity causes the injury or whether injury, by changing the normal course of metabolism, causes hyperacidity.

Incidentally it is of interest to note that the quantity of certain nitrogen compounds in plant tissues, known to be directly correlated with succulence and

vigorous vegetative development (1, 32, 39, 44), is inversely correlated with the hydrogen-ion concentration (9, 35, 44). In agreement with the latter reports, the writer found that in an experiment with young wheat plants the correlation coefficient for the nitrogen and pH values was + 0.74  $\pm$  .10; in another with older plants it was + 0.66  $\pm$  .08.

Evidently under some conditions lime decreases sap acidity, and under others it does not. Górski and Dabrowska (13) have concluded from water culture experiments that the reaction of the plant sap depends on that of the nutrient solution; but Arrhenius (2) found the pH value to be similar for tissues of wheat plants grown in nutrient solutions ranging from pH 3 to 10; Keyssner (30) found it almost the same for leaves of wheat and other plants in solutions of pH 3.8 to 7.6; and Hoagland (17) found

Table 8. pH values for the expressed juice of some highly acid tissues.

Crop	pН	Investigator	Reference
Begonia spp.	1.2 -1.7 1.3 -1.6 .9 -1.36	Rose and Hurd-Karrer Ruhland and Wetzel Smith and Quirk	Plant Physiol. 2: 449 Planta 1: 560 Phytopath. 16: 498
Bryophyllum	3.82-5.37 3.35-4.92	Gustafson Ingalls and Shive	Jour. Gen. Physiol. 7: 719 Plant Physiol. 6: 112, 117
Lemon (fruit)	2.19-2.25 2.32 2.2 2.2	Hempel  McClendon and Sharp Smith and Quirk Patten and Mains	Compt. Rend. Trav. Lab. Carlsberg 13: 117 Jour. Biol. Chem. 38: 533 Phytopath. 16: 506 Jour. Bact. 2: 221
Oxalis	1.88-2.00 1.98-2.24 1.70-1.72 1.89-2.20	Barton Fife and Frampton Smith and Quirk Rogers and Shive	Trans. Kans. Acad. Sci. 36: 73 Jour. Biol. Chem. 109: 647 Phytopath. 16: 506 Plant Physiol. 7: 233
Rumex spp.	3.19 3.75 4.03-4.51 4.0 -5.2 2.47-2.67 3.7 -3.99 3.53-3.56	Chibnall and Grover Domontovitsch	Biochem. Jour. 20: 112 Jour. landw. Wiss. (Moscow) 2: 707 Plant Physiol. 6: 109, 117 Protoplasma 17: 303 Plant Physiol. 7: 233 Pflanzenbau 6: 172 Phytopath. 16: 506
Succulents	3.95-5.59	Hempel	Compt. Rend. Trav. Lab. Carlsberg 13: 120-122
	4.1 -4.7	Czech	Bull. Soc. Bot. Genève 23 (II): 452

that it remained quite constant in barley in spite of wide variations in the reaction of the solutions. Hoagland and Davis (18) found that the pH value for Nitella cells was approximately constant at 5.2 in solutions ranging from pH 5 to 9. Theron (48) also reported that the reaction of juice expressed from the tops of plants is not directly influenced by the reaction of the culture medium. Nightingale et al. (43) found acidity to be increased usually by calcium deficiency, but Newton (40) and Dustman (10) found no appreciable increase. Truog and Meacham (50), Bauer and Haas (4) and Haas (14) found that the acidity of most of their crops was decreased by liming the soils, although a few were not. Truog and Meacham noted that one of the exceptions was the blue lupine which was sometimes injured by liming; hence they raise the question as to whether or not injury may result in an increase in acidity.

The large effect of lime on the reaction of the juice of wheat grown by Loehwing (33) on an acid humus soil, where it was in poor condition, is significant in this connection. Loehwing points out that the mineral nutrition of the plants was inadequate. Their pH values, often as low as 5.5, would in themselves indicate abnormality, according to the results of the present investigations. As Loehwing recognized, the great reduction in this acidity when the soil was limed, often to about pH 6.5, reflects a low buffer capacity resulting from inadequate mineral nutrition. Significantly, these limed plants, unlike those of the present investigation, eventually became chlorotic and unhealthy, and their acidity then increased. Loehwing concludes therefore that the extremes of high and low soil acidity may both result in hyperacidity of the sap. Clevenger (8) and Bystrikov (6) also report instances of sap acidity increased by lime.

It thus seems likely that the contradictory results reported in the literature on the effect of the reaction of the substratum on that of the plant sap may be explained on the basis of particular effects on growth. For example, wheat will not make its best growth at soil acidities greater than that indicated by a pH value of 6.0 (3, 5, 34); so liming soils more

<sup>4</sup> Optimum growth of wheat in nutrient solutions more acid than pH 6.0 has been reported, but such results may be due to reduced growth from iron deficiency in the less acid solutions (29).

acid than pH 6.0 can be expected to improve the condition of wheat and decrease its acidity, unless chlorosis or other injury occurs. But when the soil reaction is optimum for a given crop, and mineral nutrition adequate, and if vigor is not affected by change in other soil conditions, apparently no effect of lime on sap acidity is to be expected.

#### SUMMARY

pH values of the expressed juice of leaves of normal, vigorous wheat plants during the vegetative period ranged from 5.85 to 6.38 but were usually between 5.9 and 6.2. During the maturation period acidity increased, the pH value generally falling to about 5.5, rarely as low as 5.3.

Unhealthy plants became prematurely acid, but none reached pH values lower than 5.3.

Both high temperatures (above 20°C.) and lengthened daily light periods (17 hours) tended to increase acidity under experimental conditions, accounting for the abnormal acidity of spring-sown winter wheat, which grows poorly and fails to head.

The extent to which high temperature increased acidity depended on the extent to which it injured the plants. Thus acidity at the higher temperatures was determined by the temperature requirement of the particular variety.

There was no significant varietal difference in the pH values for plants grown under conditions equally favorable for normal development.

The only environmental factor found to reduce acidity was a shortened daily light period (8 hours) which favored vigorous vegetative development.

pH values for week-old seedlings grown in the dark averaged slightly lower than those for seedlings grown in the light, but the small differences are of doubtful significance.

Liming the soil had no effect on the juice acidity in these experiments, presumably because there was no effect on vigor of growth.

A compilation of reports from the literature for other plants shows a range from near pH 1 for Begonia, to near pH 7 for occasional samples of the least acid crops, with the majority between pH 5.5 and 6.5.

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# THE EFFECTS OF MAGNESIUM AND CALCIUM ON "WHITE TIP" OF RICE 1

# Alan L. Martin

A PHYSIOLOGICAL disorder known as "white tip" is frequently observed in field rice during the midseason and late growing periods. Mild forms of this disease are characterized by the appearance of white chlorotic areas at the tips of one or more of the new leaves of the plants. In more advanced stages the chlorotic area moves down the leaf, usually involving about half the structure, and a half inch or more of the leaf tip dies and dries up. When the flag leaf is injured, the panicle sheath remains very tightly rolled so that the head is compressed as it emerges from the boot. The sheath is frequently so tightly twisted that the head has to force its way through the side in order to emerge. The few flowers which are borne on heads that emerge in this manner are often sterile or produce distorted grains, resulting in decreased yields.

The similarity between these symptoms and the chlorosis described for magnesium deficiency in oats, wheat, and tobacco (Dickson, 1918; Hartman and Powers, 1928; Moser, 1933; Garner et al., 1930) led to the investigation of the effects of various magnesium concentrations on rice. It was hoped to determine whether magnesium deficiency would produce a condition similar to the disease known as white tip, whether the addition of magnesium to the growing medium would correct this condition, and whether there was an optimum magnesium:calcium ratio. Interest in the study of the effects of calcium on rice was increased by the common belief among growers that lime is detrimental to the crop.

Soil cultures in the greenhouse.—Methods and experiments.—Dark phase Crowley clay, which had produced a large percentage of white tip the previous year, was obtained at the Beaumont Rice Experiment Station. The soil was air dried for two weeks, being mixed and turned repeatedly. Eight kilograms of dry soil were placed in each of the 3-gallon glazed stoneware crocks used as culture vessels. Crocks of soil to which neither magnesium nor calcium had been added were used as controls. Magnesium, as magnesium chloride (Mg Cl<sub>2</sub> · 6H<sub>2</sub>O), was supplied in solution to a series of cultures in concentrations of 3, 9, 27, 81, 243, and 486 ppm. A like series using calcium, as calcium chloride (Ca Cl<sub>2</sub>), was set up. In the remaining series these quan-

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tities of the two salts were arranged so as to give magnesium:calcium ratios ranging from 1:1 to 1:81 and calcium:magnesium ratios in a range between 1:1 and 1:162. All concentrations were calculated on the basis of the dry weight of the soil, and the solutions were allowed to dry in the soil before planting. Ten rice seeds of the Early Prolific variety were planted in each crock, and the soil was kept damp. The crocks were placed on tables in the greenhouse and their relative positions changed weekly. When the seedlings were about five inches high, all but the five healthiest plants were removed, and a 2-inch flood of water was put over the soil. This depth of water was maintained until harvesting, with the exception of one 5-day "dry-up" to eliminate premature yellowing of the leaves. The cultures were run for a period of 18 weeks, and dry weights of the straw and of the seeds were obtained after harvesting.

Results.—The soil used in these experiments had previously produced severe white tip injury on rice plants in the field; when used in crocks in the greenhouse, white tip occurred on 40 per cent of the plants. Incipient injury appeared after ten weeks as a chlorotic white spot just below the dry brown tips of the young third, fourth, or fifth leaf. This condition spread downward until the entire leaf dried up. Before the emergence of the panicle the sheath often remained tightly rolled, and the flag leaf was stunted, thus showing severe white tip injury. The panicle was unable to emerge normally, and many flowers were injured or failed to develop; this resulted in a decreased yield of mature seeds. The plants affected by white tip were not so sturdy nor so tall as the others in the same culture. When magnesium was added to the soil cultures in concentrations of 3, 9, 27, 81, 243, and 486 ppm., the foliage of all plants was a dark green. Examples of white tip occurred when less than 81 ppm. of magnesium were used. The addition of calcium to the soil cultures in concentrations of 3, 9, 27, 81, and 243 ppm. did not produce much change in the appearance of the plants. The plants affected with white tip were light green in color, and they were all of about the same height in the different cultures. The dry weights revealed that increases in calcium supply produced corresponding increases in straw but had slight effect on seed production. When 486 ppm. of calcium was

added to the soil, the culture became slightly toxic as was shown by a decreased growth rate.

White tip occurred on the lower leaves of about 20 per cent of the plants when 3 ppm. of magnesium, with various concentrations of calcium, were added to the soil. This condition was an improvement over the cultures to which magnesium alone had been added, but there was no magnesium:calcium ratio which was distinctly beneficial with this small amount of magnesium. The addition of 9 ppm. of magnesium with varying quantities of calcium produced a greater reduction in the number of white tip leaves; however, this result depended more on the increased magnesium content than on the concentration of calcium. When 27 ppm. of magnesium and the various concentrations of calcium were added to the soil, only 4 per cent of the plants had white

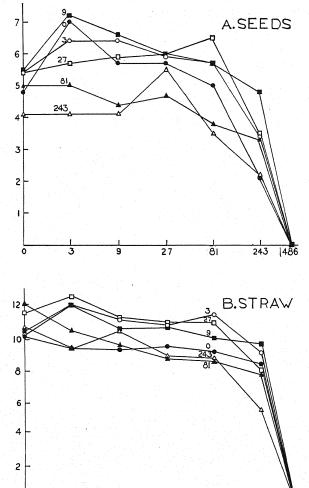


Fig. 1. A. Total weights (in grams) of seeds. B. Total weights (in grams) of straw. Each value is for 5 rice plants grown in soil cultures in the greenhouse for 18 weeks. Abscissas represent ppm. of magnesium (log scale), ordinates represent weights. Numbers on the curves indicate ppm. of calcium added to the soil of the series.

tip, and in this series there were three cultures which were completely free from this symptom. Cultures supplied with 27 ppm. of magnesium and to which 27, 81, and 243 ppm. of calcium were added produced no white tip. When 81 ppm. of magnesium were added, white tip was eliminated regardless of

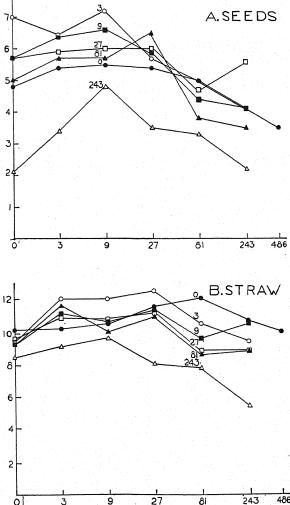
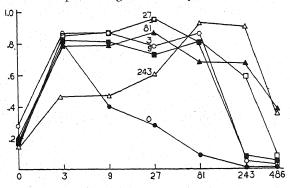


Fig. 2. A. Total weights (in grams) of seeds. B. Total weights (in grams) of straw. Each value is for 5 rice plants grown in soil cultures in the greenhouse for 18 weeks. Abscissas represent ppm. of calcium (log scale), ordinates represent weights. Numbers on the curves indicate ppm. of magnesium added to the soil of the series.

the calcium content. When both magnesium and calcium were added to the soil in concentrations of 3, 9, 27, and 81 ppm., the height of the plants was not greatly affected by the different quantities. Figure 1 shows a trend for 3, 9, and 27 ppm. of magnesium with the lower concentrations of calcium to increase the dry weights of seeds. Figure 2 shows that the addition of 3, 9, and 27 ppm. of calcium to non-toxic quantities of magnesium tended to increase the dry weight of straw and for 3 and 9 ppm. of calcium to increase the dry weight of seeds. All cultures to

which 243 ppm. of magnesium were added were free from white tip, but they showed effects of magnesium toxicity. Early death of one or more plants occurred in each culture regardless of the calcium concentration, and no seeds had ripened by harvest time. Magnesium in 486 ppm. concentration caused death to all plants before the tenth week, and the addition of calcium hastened death.

Discussion.—The addition of magnesium chloride to soil which had previously produced white tip caused the plants to grow normally. The use of low



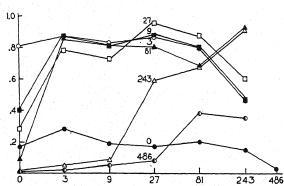


Fig. 3 (above). A. Mean weights (in grams) of tops. Each mean is from 10 rice plants grown in solution cultures in the greenhouse for 8 weeks. Abscissas represent ppm. of magnesium (log scale), ordinates represent weights. Numbers on the curves indicate ppm. of calcium in the series.

Fig. 4 (below). Mean weights (in grams) of tops. Each mean is from 10 rice plants grown in solution cultures in the greenhouse for 8 weeks. Abscissas represent ppm. of calcium (log scale), ordinates represent weights. Numbers on the curves indicate ppm. of magnesium in the series.

concentrations of magnesium reduced the number of cases of white tip, and the addition of 81 ppm. of this element completely eliminated the injury. The application of 243 ppm. of magnesium was toxic and 486 ppm. was lethal. Plants grown in this soil were uniformly light green in color unless a sufficient quantity of magnesium was present. Since magnesium is one of the constituents of chlorophyll, its absence may induce this chlorosis (Willstatter, 1909; Mameli, 1915). The characteristics of the white-tip condition lead to the assumption that it is closely related to the assimilation of chlorophyll forming ele-

ments. When the flag leaf was affected, seed vield was decreased. This may have resulted from the reduction of photosynthetic tissue during panicle formation as well as injury to the head by the pressure of the tightly whorled boot. Plants severely affected with white tip become stunted, although they reach maturity. When magnesium deficiency is overcome. the general tendency is to increase the yield of seed. Dickson (1918) reported similar results with oats. The addition of calcium salts in concentrations as great as 243 ppm. had no noticeable effect on the white-tip condition. Calcium in concentrations of 3. 9, and 27 ppm. had a tendency to increase straw production. None of the magnesium calcium ratios employed in these experiments could be considered to show optimum growth, regardless of concentration. This corresponds to the conclusions drawn by Lipman (1916) and Moser (1933). Although there were several cases in which magnesium injury was reduced by the addition of calcium to the soil cultures, these were not consistent or of sufficient degree to show clear evidence of antagonistic action.

Solution culture experiments.—In order to control nutrient conditions more effectively, water culture experiments were run in the greenhouse. It was hoped that if the magnesium:calcium ratio could be more accurately controlled the antagonistic effects of the two elements would be more pronounced.

Methods and experiments.—Rice seeds of the same stock as that used in the soil experiments were hulled by rolling on a rubbing board with a pigskin covered block. The seeds were then sterilized by soaking for five minutes in a 1:1000 solution of bichloride of mercury. Following a thorough washing to remove the disinfectant, they were placed in distilled water and kept in an incubator at 35°C. for 96 hours. At the end of this period, practically all the embryos had roots from 5 to 7 mm. long and coleoptiles about 2 mm. in length. Selected seedlings of a uniform size were placed root downward on paraffined bobbinet stretched over enamelware pans filled with distilled water and were allowed to grow in the greenhouse for eight days.

The culture vessels were 2-quart Mason jars covered with brown wrapping paper to shield the roots from the light. Each jar was fitted with a paraffined cork stopper having four holes to accommodate the plants. Young plants 7 cm. tall were placed in the corks and held in position with small tufts of cotton. The relative positions of the cultures were changed weekly.

The basic nutrient solution contained the following salts: ammonium nitrate, 0.001 M; potassium dihydrogen phosphate, 0.001 M; sodium sulfate, 0.001 M. Iron (as ferric nitrate in citric acid solution) was added weekly in sufficient amounts to maintain a concentration of 0.00001 M in the solution. The minor elements were supplied in the concentrations suggested by Trelease and Trelease (1935). The magnesium and calcium concentrations and ratios were the same as those used in the soil-

culture experiments. The solutions were prepared with water from a tin-lined still, and the solution level in each culture vessel was kept nearly constant by adding distilled water every other day. The reaction of each solution was adjusted to pH 6.5 by adding sodium hydroxide, and all solutions were renewed during the fifth week. The height and dry weight of the tops and length and dry weight of the roots were obtained at the end of the eight weeks' experimental period.

Magnesium and calcium deficiency and toxicity.— The reaction of rice plants to the absence of magnesium and of calcium in nutrient solutions was noted after the second week. Evidence of magnesium and calcium deficiencies consisted of a yellowish chlorosis followed by death and a uniform browning of most of the leaves. A few plants had leaves still living after the eight-week period, although they were greatly stunted. Figures 3 and 4 show the dry weights for these series as well as for the magnesium-free and calcium-free series. The addition of 3 ppm. of magnesium to the solution brought about a sharp increase in growth (fig. 3), but further additions (9, 27, and 81 ppm.) reduced it. The presence of magnesium changed the type of chlorosis from yellowish to whitish. With 3 ppm. of magnesium some of the leaf tips were white with whitish stripes between the larger veins, extending toward the base and closely resembling the early stage of white tip. This chlorosis became less frequent as the concentration of magnesium was increased. Concentrations of 243 ppm. and 486 ppm. of magnesium retarded growth, and all the plants were dead by the second week after being placed in the solution. The only leaf which emerged was so tightly compressed in the leaf sheath that it resembled a spiral of fine wire. This symptom is similar to that described for magnesium toxicity in wheat by Trelease and Trelease (1931).

The addition of calcium to the magnesium-free nutrient solution did not change the yellow chlorosis or appreciably benefit the growth of the rice seedlings. Another type of leaf injury made its appearance in the culture receiving 9 ppm. of calcium, and it became progressively pronounced as this element was increased. During the second week small yellow specks appeared along the margins of the lower leaves and soon involved the entire edge of the leaf, due to enlargement and coalescence of these spots. As the leaf edges died, the spots showed dark brown against a light brown background. In the more severe cases (243 ppm. and 486 ppm. of magnesium) the entire plant became affected. This leaf injury is similar to a type frequently seen in the field during the early part of the growing season, but which is not associated with white tip. In the cultures to which 486 ppm. of calcium were added, 75 per cent of the plants died by the fourth week.

Interaction of magnesium and calcium.—In order to study the effects of calcium on magnesium deficiency and toxicity and the counter-action of magnesium on calcium toxicity, six series of cultures containing varying concentrations of both salts were used. When the cultures contained 3 ppm. of magnesium, the addition of 3, 9, 27, or 81 ppm. of calcium had very little effect on growth or chlorosis. The stunting caused by 9 ppm. of magnesium was overcome by the addition of 3 ppm. of calcium, but the chlorosis occurred occasionally, and 9, 27, or 81 ppm. of calcium produced no further benefits. The addition of 3, 9, 27, and 81 ppm. of calcium to 27 ppm. of magnesium overcame the stunting observed in the calcium-free cultures. The best yields and healthiest plants for the entire experiment were in cultures containing 27 ppm. of each salt. When 243 ppm. of calcium were added to any of the above mentioned magnesium concentrations, a decrease in growth occurred. The stunting produced by calciumfree cultures having 81 ppm. of magnesium was checked by the initial addition of calcium, the greatest improvement occurring with the highest concentration (243 ppm.) of calcium. Cultures containing 243 ppm. of magnesium were toxic to the rice plants, and the additions of 3 ppm. and 9 ppm. of calcium had little effect. Magnesium toxicity was progressively decreased as 27, 81, and 243 ppm. of calcium were added. Magnesium in concentrations of 486 ppm. was toxic in all cultures, and the addition of calcium produced little improvement, although a slight increase in growth occurred in the few plants still living after eight weeks in the cultures to which 81 ppm. and 243 ppm. of calcium had been applied. Since quantities of more than 3 ppm. of magnesium caused progressively greater stunting in calciumfree cultures, it may be assumed that 3 ppm. of magnesium is sufficient to overcome magnesium deficiency in rice seedlings and that larger quantities are toxic in the absence of calcium. The addition of calcium completely antagonized this toxic action, producing growth as great as that in the cultures containing non-toxic amounts of magnesium. No definite magnesium:calcium ratio produced optimum antagonism as evidenced by maximum growth in each series. Magnesium:calcium ratios of 1:1 and 1:3 always produced the best results, but neither was consistently superior throughout the experi-

The most severe calcium injury observed in magnesium-free cultures consisted of stunting and typical brown leaf spots. The stunting was corrected by the smallest addition of magnesium except in the 243 ppm. of calcium series. The characteristic leaf spots, which made their appearance with 9 ppm. of calcium and were even more common with 27 ppm., were absent when 3 ppm. of magnesium were added. The more severe injury caused by 81 ppm. and 243 ppm. of calcium required proportionally high concentrations of magnesium to produce benefits. Injury due to 81 ppm. of calcium was eliminated only in the presence of 243 ppm. of magnesium. The leaf spots produced by 243 ppm. of calcium were never entirely overcome, but the stunting was greatly reduced by the use of larger quantities of magnesium so that the yields with 81 ppm. and 243 ppm. of

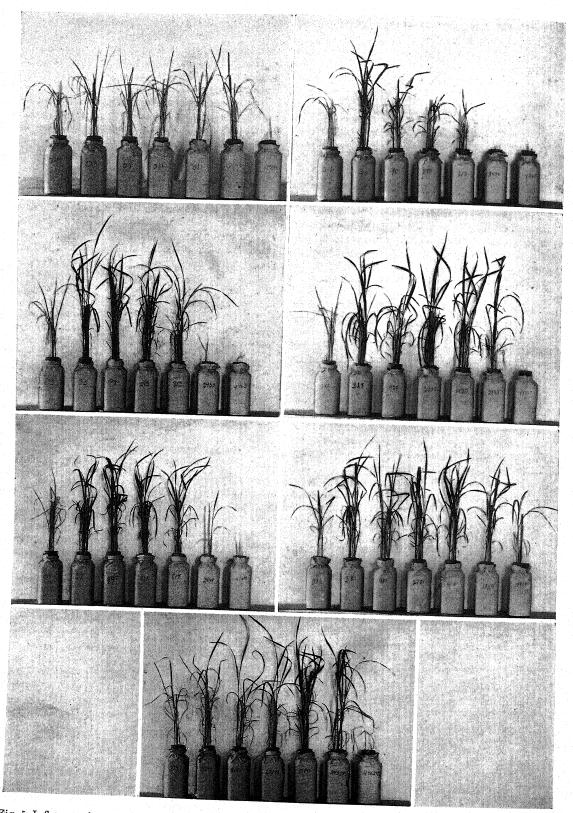


Fig. 5. Influence of magnesium and of calcium on rice plants grown for 8 weeks in the greenhouse. The series at the upper left had no magnesium, and the calcium content varied from 0 ppm. to 486 ppm. The series at the upper right had no calcium, while the magnesium content varied from 0 ppm. to 486 ppm. The rest of the series have 3, 9, 27, 81,

magnesium were next to the best obtained in the experiment. The photographs in figure 5 show the effects of magnesium on calcium toxicity. There was very little change in growth brought about by increases in the calcium concentration in magnesium-free cultures. The best growth occurred when 3 ppm. of calcium were in the solution; higher concentrations reduced growth and caused leaf injury. The corrective value of magnesium for the leaf spots and the tendency to increase yields showed that the highest degree of antagonism takes place when the magnesium:calcium ratio is 1:1 or 1:3.

Dry weights of roots showed that their development under the various concentrations and ratios of magnesium and calcium corresponded to the results obtained from the dry weights of plants. No specific injury was evident on the roots at the time of har-

vesting.

Discussion .- Rice plants were sensitive to changes in concentrations and ratios of magnesium and calcium in culture solution. In the absence of both elements the plants grew very little, the leaves died early and turned a uniform brown color. The response to 3 ppm. of magnesium, without calcium, produced a good yield, although whitish chlorosis was prevalent. Successive increases of magnesium, in calcium-free solutions brought about reductions in growth, and death occurred with 243 ppm. of magnesium. In the absence of magnesium, the addition of calcium in quantities varying from 3 ppm. to 486 ppm. did not increase growth, and the characteristic leaf injury due to excess calcium became more conspicuous as the concentration increased above 9 ppm. of calcium. The benefits derived from calcium in the absence of magnesium were so questionable that it appears that calcium is beneficial only when magnesium is present (Loew, 1903). When both magnesium and calcium were present in the culture solutions, growth increased and leaves were healthier in appearance. The best growth with complete absence of leaf injury occurred in cultures containing 27 ppm. of magnesium and 27 ppm. of calcium. The cultures containing 81 ppm. and 243 ppm. of magnesium plus 243 ppm. of calcium closely approximated these. This indicates that water cultures containing magnesium and calcium in the ratio of 1:1 or 1:3, when both salts are in concentrations of 27 ppm. or more, produce maximum growth during the early growing period of rice plants. The conclusions drawn by Aso (1904) working with rice and Loew (1903) with other cereals correspond closely with the above results. Good growth was obtained when the above ratios were used, and the concentrations of magnesium and calcium were below 27 ppm., but the white chlorosis of magnesium deficiency occurred occasionally on the leaves. In considering the good yields of the cultures containing 3 ppm. of magnesium and no calcium, it must be remembered that these plants did not have conditions of complete calcium deficiency due to the calcium content of the rice seeds themselves. As the magnesium content of the cultures was increased and the calcium content of the seed remained constant, the ratio of magnesium to calcium was increased beyond a point where benefit was possible.

The brown spots which appeared on leaves in the magnesium-free cultures containing 9 ppm. or more of calcium disappeared when magnesium was added

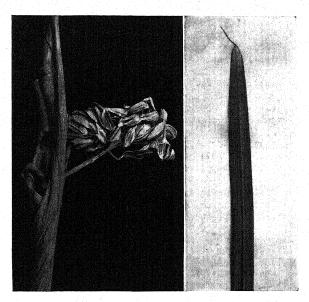


Fig. 6. Typical examples of white-tip injury to rice plants. A (left). Extreme injury showing panicle forcing its way out through tightly whorled boot whose flag leaf was severely damaged by the disease. B (right). Mild white-tip injury observed on lower leaf in culture solution deficient in magnesium.

in sufficient quantity. The reciprocal antagonism between magnesium and calcium in rice plants is shown by the reduction which each of these elements makes in the toxic effects of the other and the stimulus to growth when both are present (Miyake, 1913).

# SUMMARY

Injury observed on leaves of rice plants grown in soil and water cultures, which were low in magnesium content, was similar to a chlorosis observed in the field and known as "white tip." This condition was eliminated in soil cultures when 81 ppm. of magnesium were added and in water cultures with 27 ppm. of magnesium. However, these concentrations of magnesium produced toxic effects, such as stunting and decreased yields, and normal plants were obtained only when calcium as well as magnesium was supplied to the cultures.

Calcium was not toxic in low concentrations in soil cultures. In magnesium-free water cultures 9 ppm.

and 243 ppm. of calcium, respectively, with magnesium varying in each series, reading from left to right, from 3 ppm. to 486 ppm.

or more of calcium produced stunting and brown leaf spots. These injurious effects were not evident when sufficient magnesium had been added.

The greatest benefit from the antagonistic action between magnesium and calcium occurred with the magnesium:calcium ratios of 1:1 or 1:3 when calcium and magnesium were present in concentrations of 27, 81, or 243 ppm. of each element.

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# MITOSIS IN LEAVES OF SMILAX 1

# Bernice M. Speese

This is a preliminary report on nuclear divisions in leaves of Smilax L. Two species native at Williamsburg, Virginia, were investigated: S. rotundifolia L. and S. Bona-nox L. The method of preparing the leaves for study was essentially that of Baldwin (1939): fixation for at least twelve hours in Carnoy's fluid, treatment for four minutes with a solution of equal parts 95 per cent alcohol and concentrated hydrochloric acid, and, prior to smearing in iron aceto-carmine, transference again into Carnoy's. The preparations were sealed with a gum mastic-paraffin mixture. Reasons for studying Smilax were several: the leaves are glabrous; they smear well; the chromatic mass is large, and dividing nuclei are, therefore, readily observable. The stage in prophase when the chromosomes are first easily recognizable and the stage in telophase when the chromosomes are still apparent were used as arbitrary limits of mitosis.

The 2n-chromosome numbers of S. rotundifolia (fig. 1) and of S. Bona-nox (fig. 2) were found at leaf metaphase to be 32; the sex of the various plants was not known. (Herbarium specimens of the species will be distributed under the numbers: Baldwin 416 and 417.) The chromosomes in both species vary considerably in size and morphology. Jensen (1937) reported an n-number of 16 for S. rotundifolia from the Blue Ridge Mountains in North Received for publication September 2, 1939.

The writer expresses appreciation to Dr. J. T. Baldwin, Jr., for suggesting and criticizing this work.

Carolina. He interpreted certain of his observations to "suggest a record of previous hybridization for the species." It is of consequent interest to note that counts from leaf mitoses (sometimes considered probably to be chromosomally aberrant) of plants in the Virginia coastal plain corroborate the gametic number determined for supposed hybrid plants in the North Carolina mountains. Other chromosome numbers reported for *Smilax* are:

	2n	$\boldsymbol{n}$	
S. China L		30	Nakajima (1937)
S. glauca L		14	Jensen (1937)
S. hederacea L var. nipponica	30	••	Nakajima (1937)
Maxim.			
S. herbacea L		12-13	Elkins (1914)
		12	Humphrey (1914)
		13	Lindsay (1929; 1930)
S. Oldhami Miq	30		Nakajima (1937)

Of the above workers only Nakajima (1937) got evidence of heteromorphic chromosomes; he reported sex chromosomes of the X-Y type in three species.

Young leaves of S. rotundifolia were used for the determination of the number of nuclear divisions in process for a given area. A leaf of the size shown in figure 3 was fixed at 4:30 p.m. on July 3, 1939; a total of 31,380 mitoses was found in Section A of that leaf; all the tissues included dorsiventrally in the section were examined. There was a general de-

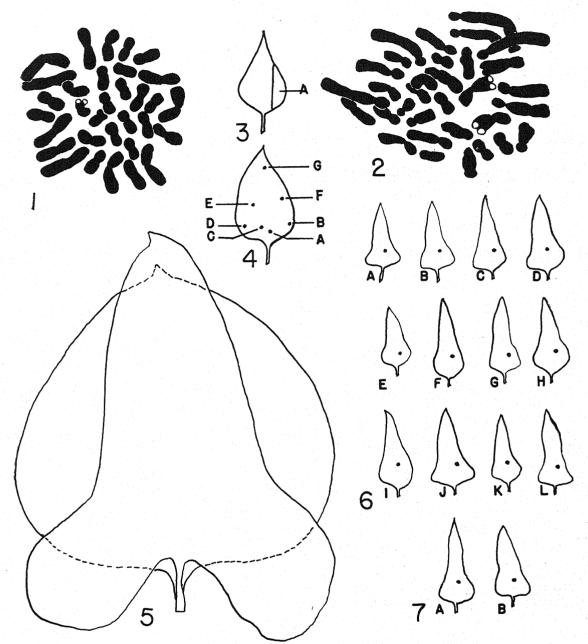


Fig. 1-7.—Fig. 1. Leaf metaphase of Smilax rotundifolia L., 2n=32. Magnification ca.  $3800\times$ .—Fig. 2. Leaf metaphase of S. Bona-nox L., 2n=32. Magnification ca.  $3800\times$ .—Fig. 3. Natural size outline of S. rotundifolia leaf with area in which 31,380 mitoses were counted.—Fig. 4. Natural size outline of S. rotundifolia leaf of which samples A-G were studied cytologically.—Fig. 5. Natural size outlines of mature leaves of S. rotundifolia and S. Bona-nox.—Fig. 6. Outlines of twelve leaves of S. Bona-nox fixed one each at 2-hour intervals throughout a 24-hour period; places from which samples were taken for division counts are indicated.—Fig. 7. Outlines of two other leaves of S. Bona-nox studied.

crease in mitotic frequency from the proximal to the distal part of that area. Comparable results were obtained from random samples, about one millimeter in diameter, punched from a leaf of the size represented in figure 4; the leaf was fixed at 3:00 p.m. on July 24, 1939; the number of divisions for each of the samples designated in figure 4 was determined: Sample A, 1952; B. 2012; C, 2166; D, 1787; E,

2089; F, 1469; G, 92. It is apparent that the results for Sample E are somewhat "off," but, in general, the basal region appears to be most mitotically active. Leaf shape is dependent upon the frequency patterns of mitosis. Karyokinesis is fundamental to growth and morphogenesis. It would be of some significance to determine for whole leaves the spatial mitotic frequencies and to compare in this regard

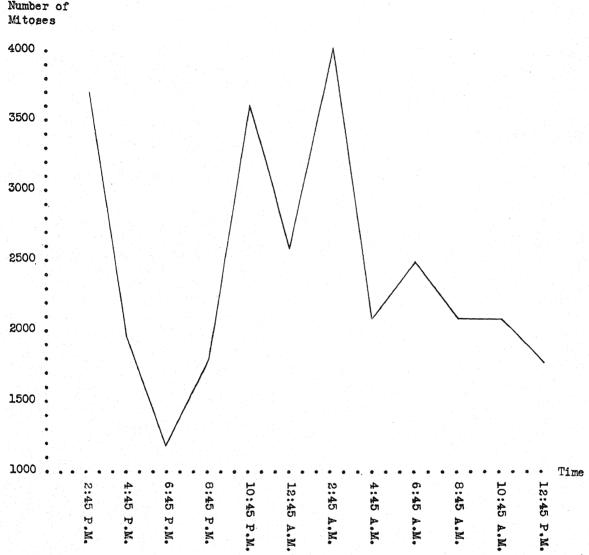


Fig. 8. Curve of division frequency in S. Bona-now leaves fixed one each at 2-hour intervals throughout a 24-hour period.

leaves which differ in shape, as do, for example, the mature leaves of S. rotundifolia and S. Bona-nox (fig. 5).

The literature, reviewed by Friesner (1920), indicates that mitotic divisions occur in waves and are rhythmic. Though, because of the peculiar physiology of leaves, pronounced fluctuations in their division curves might be expected, no analysis seems to have been made of the mitotic rhythms in these plant organs. An approach to this problem was attempted in the present work. Twelve S. Bona-nox leaves of approximately the same size were fixed, one each at 2-hour intervals throughout a 24-hour period on August 2-3, 1939, and smears made of one-millimeter-in-diameter samples, punched from a similar area, located by veins, on each leaf (fig. 6). Since only one young leaf of a given size usually occurred on a single plant of S. Bona-nox, twelve different plants

(growing at the edge of the William and Mary campus) were used. The number of nuclear divisions was counted for each of the samples: Sample A, 2:45 p.m., 3734; B, 4:45 p.m., 2003; C, 6:45 p.m., 1248; D, 8:45 p.m., 1756; E, 10:45 p.m., 3615; F, 12:45 a.m., 2559; G, 2:45 a.m., 3978; H, 4:45 a.m., 2064; I, 6:45 a.m., 2521; J, 8:45 a.m., 2149; K, 10:45 a.m., 2143; L, 12:45 p.m., 1846. These data produce a curve (fig. 8) with two minima-6:45-8:45 p.m. and 12:45 p.m.—and with three maxima—primary maxima, 12 hours apart, at 2:45 p.m. and 2:45 a.m., and a secondary maximum at 10:45 p.m. Since it happened that the leaves fixed at 10:45 p.m. (fig. 6E) and at 10:45 a.m. (fig. 6K) were smaller than the others, two additional leaves of a more comparable size were fixed at these hours on August 17 and August 18, respectively (fig. 7). The number of divisions for the second 10:45 p.m. leaf (fig. 7A)

was considerably lower than for the first (fig. 6E), 3141 as compared with 3615, yet high enough to constitute a marked maximum in the curve. The number of divisions in the second 10:45 a.m. leaf (fig. 7B) did not differ significantly from the number in the first (fig. 6K), 2255 as compared with 2143. These observations on mitotic fluctuations in leaves of Smilax are preliminary, but they support a conclusion reached by Friesner (1920) for roots of several different genera: "the curve of cell division in all plants studied exhibits a number of oscillations in the 24-hour period, in the majority of plants three." Likewise, Ono (1937) found in root tips of Crepis "two marked dividing periods."

#### SUMMARY

Smilax rotundifolia L. and S. Bona-nox L. both have 32 chromosomes at leaf metaphase. Determinations were made from smears.

The number of dividing nuclei was counted for certain selected areas of young leaves of S. rotundifolia. A general reduction in mitotic frequency appears to occur proximo-distally in those leaves.

A temporal frequency established for mitosis in leaves of S. Bona-nox gives a curve with two marked minima and three maxima.

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# ROOT ACTIVITY AND THE OXYGEN REQUIREMENT IN RELATION TO SOIL FERTILITY 1

### Kenneth S. Karsten

LIFE MAY be regarded as an unstable equilibrium since energy is involved in the maintenance of this equilibrium. This energy may be furnished directly by the light of the sun or indirectly by the oxidation of carbohydrates or other substances capable of yielding energy upon undergoing oxidation. The importance of the energy relationships of a number of life processes in plants has been realized, and work has been carried out in this field by a number of investigators.

Since the amount of energy released during aerobic respiration of most substances is much greater than that released during anaerobic respiration of the same substances, the factors that influence aerobic respiration are of great importance in any consideration of process-energy relations. Factors that most commonly limit the rate of aerobic respiration are the oxygen supply and the carbon-dioxide content of the medium and the amount and availability of food material.

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The writer wishes to express his appreciation to Dr. B. M. Duggar for helpful suggestions and criticism offered during the carrying on of this work under his direction in the Botany Department, University of Wisconsin, Madison, Wisconsin. Appreciation is also expressed to Dr. H. G. Petering for acquainting the writer with the use of the dropping mercury kathode and to Mr. D. M. Batson for the gift of a number of soil samples.

Aerobic respiration is necessary for growth in most forms. Allison and Shive (1923) have shown that aeration of sand or nutrient solution cultures of soybeans increased the growth of both roots and tops. Loehwing (1934) showed that the soil atmosphere is normally an important factor in the developmental cycle.

Richards (1938) has given evidence that a given rate of respiration can maintain only a definite amount of protein. Henderson (1934) concluded that the absorption of water by corn roots is accompanied by the expenditure of energy. Mason and Phillis (1936) believe that a part of the energy released during respiration must in some way expedite transport. Many of the workers on the problem of salt absorption and accumulation conclude that respiration determines absorptive activity (see Hoagland, 1937).

The extent and activity of the root systems of plants govern the size and productivity of the aerial parts to a great degree. Root activity is controlled by the character of the soil. The physical, chemical, and biological nature of the soil is being constantly modified because of (1) the presence of living organisms in the soil, (2) the presence of decomposable substances, and (3) the action of external influences. Soil is a dynamic and not a static system,

and changes in the nature of the soil are reflected in the growth of the plants which it supports.

A large number of factors determine soil fertility by their direct or indirect action on the roots of plants. Among these factors are compactness of the soil, pore space, water content, water-holding properties, presence and availability of mineral nutrients, organic content, nature of the organic matter, character and amount of micro-organisms, composition of the soil gases, pH of the soil solution, presence or absence of substances that are toxic to plants, and temperature. A study of soil fertility is difficult, since no single factor can be controlled without changing many or all of the properties of the soil.

The amount and character of the organic matter in the soil are often factors that determine the activity of soil micro-organisms. The oxygen uptake by soil micro-organisms invariably increases with increase in their actiity. As a result, the oxygen content of the soil is lowered and the carbon-dioxide content is raised. Soil temperatures also determine the rate at which micro-organisms lower the oxygencontent and raise the carbon-dioxide content of the soil. If conditions are such that the micro-organisms become so active as to impose anaerobic conditions on the soil, the roots of most plants will be adversely affected by (1) lack of oxygen, (2) high carbondioxide content, and (3) products of incomplete metabolism produced by the micro-organism (see Breazale, 1924; Conrad, 1927).

Poor growth is characteristic of most crops growing under anaerobic soil conditions. The amount of oxygen contained in the soil depends on various soil characteristics. Hutchins (1926) found that the oxygen-supplying power of the soil varies with moisture content and compactness of soil, loosely packed dry soils having the highest oxygen-supplying power.

The amount of literature on the rôle of oxygen in root activity is large. The work accomplished during the period 1668 to 1921 has been reviewed by Clements (1921). The following statement made by Clements in his review is of great interest: "The essential features of the respiration of plants were established in the short period from Ingenhousz (1779) to Saussure (1804). It was shown not only that all organs of the plant possessed this function in common, but also that roots respired in exactly the same manner as stems and leaves, despite the difference in medium. This is supported by practically all the evidence drawn from germination, since the early stages of this have to do with the radicle. In consequence, there is a complete chain of evidence from Mayo (1668) to the present time as to the necessity of oxygen for root activity. The direct proof of this has been obtained by showing the use of oxygen by roots, and this has been confirmed again and again by their behavior in the absence of oxygen."

The work of Boynton, et al. (1938), on the effect of oxygen concentration on apple tree root activities gives us some insight with regard to the importance of oxygen as a factor in soil fertility. They state that when the oxygen in the soil atmosphere is maintained at 3 per cent, the roots are at a subsistence level with regard to oxygen—that is, they continue to live but grow slowly if at all. These workers report that absorption and accumulation were probably inhibited at oxygen pressures below 10 per cent.

Lundegårdh and Burström (1935) studied quantitatively the respiratory activity of the roots of wheat seedlings. From the results of their experiments they concluded that the respiratory activity consists of two parts, the basal respiration, which is necessary for maintaining the roots and for growth, and the anion respiration, which furnishes the energy required for the absorption of anions. They state that the basal respiration is always aerobic and that under no conditions was there any evidence of anaerobic basal respiration. The anion respiration can be anaerobic.

It is readily seen that if the oxygen content of the soil is reduced to such an extent that the rate of basal respiration is radically lowered, the roots of the plants will die, and death of the tops will ensue. If the reduction is not sufficient to cause death but is sufficient to impair the growth of the roots, this condition will be reflected in reduced growth and reduced productivity of the aerial portions of the plant.

Experimental methods and materials.—Since no simple method was available for the quantitative measurement of the oxygen content of soils, the polarographic method of analysis was adapted to this use. The method to be described for the meassurement of the concentration of dissolved oxygen is a modification of the polarographic method, shown by Vitek (1935) to be applicable to the determination of oxygen in solution. The modification used is that of Petering and Daniels (1938). The instrument may be constructed from ordinary laboratory materials and will be referred to as the dropping mercury kathode. See Kolthoff and Lingane (1939) for a review of the theory and applications of the polarographic method of analysis.

Although theoretical interpretations of the polarographic method have been proposed, it may be regarded as an empirical procedure that has been checked against standard methods of chemical analysis. Its use depends on the fact that solutes are reduced or oxidized when a current is passed through a solution at voltages above the decomposition potential. The amount of current flowing depends on the concentration of the reducible material in the solution.

The dropping mercury kathode gives reproducible current-voltage curves because a fresh electrode surface is being exposed continuously. At low voltages, a small residual current flows until the decomposition potential is reached. Then, as the voltage is increased, the current depends directly on the voltage. As the voltage is still further increased, a point is reached after which the current is limited by the rate of diffusion of the reactant to the surface of the mercury drop. The current-voltage curve then be-

comes flat in the ideal case, the current remaining constant until the voltage becomes high enough to produce some other chemical reaction at the kathode. An ideal current-voltage curve is plotted in figure 1.

The portion of the curve OA represents the residual current. At A the decomposition potential is reached. In the region of the curve labeled AB the limiting factor is the rate at which electrons having sufficient energy reach the kathode, the rate of diffusion being much greater than the supply of electrons having the proper energy. At B the current becomes limited by the rate of diffusion of the reducible material to the kathode surface. It may be seen that the portions of the curve, OA and BC, are parallel, and the difference in current B between these two parallel portions of the curve is a function of the concentration of the reducible material in the solution.

Vitek (1935) described the electrode processes for oxygen as occurring in two steps, (1) the reduction of oxygen to oxygen ion, and (2) the reduction of the oxygen ion to hydroxide ion. The first reaction has been found to occur at about 0.1 v and is represented in figure 1 by the portion of the curve that extends from O to C. The second reaction occurs at about 1 v, and the portion of the curve CD represents the beginning of the curve for this reaction.

The apparatus used is represented diagrammatically in figure 2 and is similar to that used by Peter-

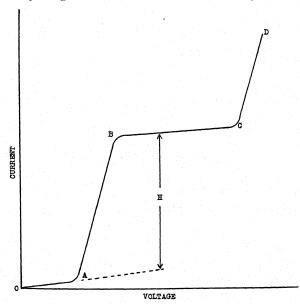


Fig. 1. An ideal current-voltage curve.

ing and Daniels (1938) who showed the application of this method to the measurement of photosynthesis and respiration of algae and to the measurement of respiration of yeast, blood cells, and animal tissue.

WB represents the working battery and is the source of electromotive force for the voltage impressed across the electrodes of the dropping mercury electrolytic cell.

In order to impress a known voltage across the electrodes, a type K potentiometer was used, but a simpler type will serve as well. Large capacity standard cells that deliver the proper voltages may also be used as Petering and Daniels (1938) have suggested.

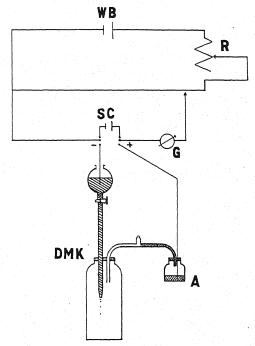


Fig. 2. Diagram of apparatus used in the soil oxygen determinations.

A Weston primary cell marked SC in the diagram is used to keep the potentiometer standardized.

The galvanometer G is of the lamp and scale type and has a sensitivity of  $2.5 \times 10^{-8}$  amps per mm.

The dropping mercury electrolytic cell is represented in the lower part of figure 2. The dropping mercury kathode DMK consists of a reservoir of mercury connected to a section of fine capillary glass tubing. The kathode is connected to the potentiometer by means of a platinum-copper glass sealed lead which is inserted into the mercury of the reservoir. The height of the column of mercury is about 50 cm. and the orifice of the capillary tip is so regulated that from one to two drops of mercury are formed per second. The capillary tip is conveniently made by drawing out thermometer tubing.

The anode A is outside the reaction cell and consists of a pool of mercury in the bottom of a small bottle that contains a solution similar to that being analyzed. The anode vessel solution is connected to the reaction cell solution by means of a KCl-agar bridge. The anode is connected to the galvanometer by means of a platinum-copper glass sealed lead. The anode has an area of about 7 cm.<sup>2</sup> The reaction vessel has a volume of approximately 150 cc.

A 0.1 N KCl solution was used as a medium for the dissolved oxygen. The solution also contained 0.05 per cent gelatin to prevent an absorption maximum and 0.05 per cent thymol to prevent loss of oxygen that might be caused by the activities of micro-organisms.

In these calibrations the oxygen concentration of a solution is determined by sodium thiosulfate titration using the standard Winkler method with man-

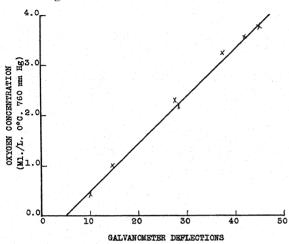


Fig. 3. Oxygen calibration curve for the dropping mercury electrolytic cell used.

ganous sulfate and potassium iodide. Various concentrations of oxygen are obtained by saturating the solution with air and then bubbling nitrogen through this for varying lengths of time.

In using the dropping mercury kathode the solution to be analyzed is placed in the reaction vessel so as to completely fill it. A potential of 0.1 v is then impressed across the electrodes, and the galvanometer reading is noted at this potential. The galvanometer needle moves constantly, since the circuit is disturbed between the falling of one drop and the formation of the next drop. The high reading is taken as the correct reading. For example, if the galvanometer indictor moves from 70 mm. to 78 mm. and then back to 70 mm. continuously, 78 mm. is taken as the proper and correct reading. A potential of 0.9 v is then impressed across the electrodes, and a second reading is taken. The difference between these two readings (at 0.1 v and 0.9 v) is the number that is proportional to the oxygen concentration. When these numbers are plotted against the oxygen concentration as determined by the Winkler method a straight line calibration which is shown in figure 3 is obtained.

A solution of the same composition as that used in the calibration of the dropping mercury kathode is used in the determination of soil oxygen. The solution is boiled under a reflux condenser for 15 minutes to remove dissolved oxygen. The hot solution is then siphoned off into hot 150 cc. bottles provided with glass stoppers, the delivery tube being extended to the bottom of the sample bottle during this operation. The soil sample is taken with a cork borer, and a 3 gm. portion of the core thus obtained is added to

the solution contained in one of each pair of sample bottles. The stopper of the bottle to which the soil sample has been added is replaced immediately, and the bottle is agitated until all the soil gas has been dissolved. The resulting solution is analyzed for oxygen by use of the dropping mercury kathode, the sample bottle being used as a reaction vessel during the analysis. The original boiled solution is likewise analyzed for oxygen. Knowing the volume of the bottle in which the soil sample was placed, the amount of oxygen contained in the sample of soil can be obtained by suitable calculations.

The relation of soil oxygen to soil organic and nitrogen content and to the growth of wheat seedlings was determined as follows. A soil was prepared by mixing two parts of garden loam with one part of sand. To portions of this mixture were added various amounts of starch and of nitrogen as ammonium sulfate. The effects on the growth of wheat seedlings and on the oxygen content of the soil were noted. The soil was not moistened until the seedlings were planted, and all plants were grown in a constant temperature chamber provided with uniform artificial lighting. The temperature of the culture chamber was regulated at 26°C. ± 0.5°C. Intensity of illumination was maintained at approximately 100 foot candles as measured by means of a Weston light meter. Seventy-five watt tungsten filament nitrogenfilled bulbs were used. The wheat grains were germinated at room temperature in pot saucer germinators provided with distilled water. Seedlings were selected for size and planted when the coleoptile had attained a length of 15 cm.

The soil in which the seedlings were grown was contained in specially constructed flower pots which allowed the soil to be watered by a method of autoirrigation. Each pot was provided with a glass tube of 1 cm. diameter which extended upward through the drainage hole of the pot to the extent of 2.5 cm. The tube extended downward from the bottom of the pot for a length of 10 cm. The lower end of the tube was covered with a layer of muslin. The glass tube was held in place in the pot by means of sealing wax. The tube and lower part of the pot were filled with washed dry sand, and the remainder of the pot was filled with the soil under investigation. The soil and sand were wet thoroughly with distilled water, and the pot was lowered over a reservoir of distilled water so that the tube was immersed to the desired depth. With the bottom of the pot 6 cm. from the surface of the water, adequate water content (15 per cent) was maintained in the soil for good growth of the wheat plants. As water is lost from the soil more is taken from the reservoir by capillary action through the sand in the tube. The level of the water in the reservoir was kept as nearly the same as possible throughout the investigations. This type of auto-irrigated flower pot has been operated successfully during three months of continuous use and may be used with either soil or sand.

EXPERIMENTAL RESULTS.—The results of analyses on a variety of soils are given in table 1. The soils were kept in flower pots in the laboratory.

Table 1. Oxygen content of various soils (expressed as milliliters of oxygen at 0°C. and 760 mm. of mercury pressure) per kilogram of soil.

Soil	Water content	$Ml O_2/kilo of soil$
	30%	(3.142
Sandy loam		3.186
	30%	7.500
Superior clay		7.114
Garden loam	(10%	6.667
	20%	3.824
Barnes silt clay loam		4.600
Carrington silt loam		10.622
Miami silt loam		3.200

The results of experiments involving soil mixtures of varying organic and nitrogen content are given in table 2. The oxygen content of the various soil mixtures was determined on the sixth and twelfth days following planting. Growth of the wheat seedlings was measured as fresh weight of the tops per six plants. Five pots of six plants each were used with each soil mixture. Since determinations of the soil in the five pots of each series showed very little variation in oxygen content, analyses were made from one pot in each series so as to disturb plant growth in the other pots as little as possible. The roots of the plants were not sufficient in amount to affect the oxygen content appreciably. The results of this experiment are shown in table 2.

Some idea of the effect of soil oxygen content on the growth of the wheat seedlings can be obtained by plotting the results obtained in this experiment. The curve is plotted in figure 4. The oxygen content of the soil as determined on the twelfth day is plotted on the abscissa, and the growth of the wheat plants as fresh weight of the tops of six seedlings expressed in milligrams is plotted on the ordinate. Naturally some factors other than oxygen content of the soil—e.g., carbon-dioxide content—may have exerted an influence. The pH of the soil was lowered to not lower than pH 5.3 as a consequence of the addition of the starch and ammonium sulfate.

That the lowered oxygen content of the soil mixtures was caused by an increase in the activity of the soil micro-organisms was shown by the fact that such soil mixtures supported a profuse fungus growth. In nearly all cases the soil became compact and hard.

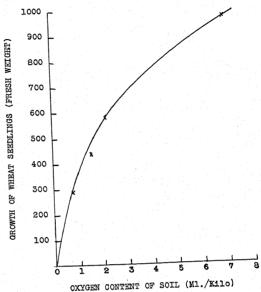


Fig. 4. Graph showing the relationship of soil oxygen content to the growth of wheat seedlings.

Discussion.—The results of these experiments show that the oxygen content of soils varies with the soil type, water content, nitrogen content, and organic content. Soil oxygen content for various soils given in table 1 are less than might be calculated on the basis of pore space and water content. The reason for this is that the composition of the soil atmosphere is modified by the soil micro-organisms with the result that usually the oxygen content of the soil atmosphere is less than that of the external air. Since the soils were kept in pots in the laboratory, the values given may vary somewhat from those that might have been obtained in soils in the field. Soils in the field, however, would be expected to vary to such a large extent that very many samples would

Table 2. Effect of organic matter and nitrogen on the oxygen-content of soil and on the growth of wheat seedlings.

Percentage starch	Percentage (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	$egin{array}{ll} O_2 ext{-Content} & O_2 ext{-Content} \\  ext{per kilo of soil.} &  ext{per kilo of soil.} \\  ext{Sixth day} &  ext{Twelfth day} \\ \end{array}$	Growth of wheat as fresh weight of tops in mgm. per 6 plants
0.00 0.00 1.67 0.33 1.00 1.67 3.93 6.67	0.00 0.33 0.00 0.33 0.33 0.33 0.33	8.961 ml 7.000 ml 3.844 ml 2.180 ml 1.102 ml 0.788 ml 4.278 ml 1.548 ml 3.000 ml 0.000 ml 1.333 ml 0.000 ml 0.000 ml 0.000 ml 0.000 ml 0.000 ml	977.6 ± 92.2 580.7 ± 30.4 290.1 ± 143.8 439.2 ± 106.9 Dead on 12th day Dead on 7th day Dead on 7th day

have to be analyzed in order to obtain a representa-

tive average value.

Added organic matter and nitrogen allowed increased growth and activity of micro-organisms which take up oxygen so rapidly under such conditions that the oxygen content of the soil is abnormally reduced. If the oxygen content is diminished sufficiently, root activity is lowered, and the entire plant suffers reduced growth. If the oxygen content of the soil becomes zero, the plants soon die.

#### SUMMARY

The dropping mercury kathode may be conveniently used for the quantitative determination of oxygen in soils. A reduced oxygen content and reduced rate of plant growth resulted from treatments of soil with starch and ammonium sulfate.

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# AMERICAN JOURNAL OF BOTANY

### GENERAL SECTION

ABSTRACTS OF THE PAPERS PRESENTED BEFORE THE GENERAL SECTION OF THE BOTANICAL SOCIETY OF AMERICA, COLUMBUS, OHIO,
DECEMBER 28 TO 30, 1939

DECOMPOSITION OF CHITIN BY MARINE BACTERIA. Charles W. Hock, Woods Hole Oceanographic Institution, Woods Hole, Mass.-Chitin-decomposing bacteria have been isolated from sea water, marine sediments, decomposing crabs, and the intestinal contents of marine animals. Although some of the bacteria grow in a medium consisting only of pure chitin in sea water, others require the addition of a readily available source of nitrogen. Acids, reducing sugars, and ammonia have been identified as decomposition products. In an attempt to simulate natural conditions strips of crude Limulus shell were put in jars of littoral sand, bottom mud, and raw sea water. Decomposition of the shell was rapid in the sand, moderate in the mud, and altogether negligible in the water. Carbon and nitrogen analyses of the shell, sand, and mud, were made at frequent intervals, in order to follow changes in the C:N ratio in the system.

OBSERVATIONS ON THE REPRODUCTION OF ULVA LOBATA. Gilbert M. Smith, Stanford University, Calif.—Daily collections were made of plants growing two feet above mean low tide level at Cypress Point near Monterey, Calif. Twice each lunar month there was a conspicuous development of fertile cells at the margins of thalli for three or four days; and then a discharge of all zooids within a day or two. The three- or four-day period of zooid accumulation is the only time in a tidal cycle when there is no tidal exposure of the Ulva during daylight. The period of profuse discharge of zooids occurs on days when exposed plants are reflooded by the tide shortly after daybreak.—In any random collection of individuals about 80% of the fertile plants produce biflagellate gametes and 20% produce quadriflagellate zoospores.-The gametophytes are anisogamous and with female gametes about twice the size of male gametes. Male gametophytes are distinguishable in the field because of their yellowish-green fertile margins. Female gametophytes have grass-green fertile margins but they cannot be distinguished in the field from fertile sporophytes.—The gametophytes are strictly heterothallic. When male and female gametes are mixed in a drop of water there is a clumping of many male gametes about a single female gamete. Both types of gametes are positively phototactic but as soon as a pair begin to fuse the uniting pair becomes negatively phototactic and swims to the less intensely illuminated margin of the drop.—Zygotes and zoospores show the beginnings of germination within two or three days. No partheno-genetic germination was observed of either male or female gametes.

FORMATION OF CELLULOSE PARTICLES IN HALI-CYSTIS SP. Wanda K. Farr, Cellulose Laboratory, Boyce Thompson Institute, Yonkers, N. Y.—The chief visible product of carbohydrate synthesis within the chloroplast is in the form of "paramylum" rings. In the young, disc-shaped plastid there are two small rings, usually arranged so that a line passing through the central axis of each ring lies approximately parallel to the major axis of the plastid disc. As the chloroplast enlarges there is a successive formation of new rings of larger diameter but equal thickness immediately inside the plastid membrane. These larger rings disintegrate within the plastid into uniform-sized particles. After the formation and particulate disintegration of two or more large rings, the chloroplast membrane itself breaks down, releasing into the outer regions of the cytoplasm the entire plastid content. This material of plastid origin goes directly into formation of the lamellae of the cell membrane. The two small rings disintegrate, each into four particles, either before the breakdown of the plastid membrane, after the breakdown of the plastid membrane, or after the deposition of the plastid material in the cell membrane. The uniform-sized particles resulting from the disintegration of the rings formed within the chloroplast are identified as mercerized cellulose by both microscopic and X-ray diffraction analyses.

Ventral specializations of the marchantiales. Marjorie McConaha, University of Iowa, Iowa City, Iowa.—The ventral specializations of six genera were measured and their relations analyzed. The absorptive surface of the thallus may be increased as much as 5480% by its scales and rhizoids. There was a definite segregation of the two types of rhizoids in all thalli examined. Smooth-walled rhizoids emerge from beneath the scales and make contact with the substrate. Regardless of scale arrangement tuberculate rhizoids form a capillary strand beneath each scale and parallel to the thallus. In all these genera the external conduction of water along the thallus is aided by the relations of the ventral organs.

NUTRITION OF THE SPOROPHYTE IN THE MUSCI. Harold C. Bold, Vanderbilt University, Nashville, Tenn., and Barnard College, Columbia University, New York, N. Y.—Until recently the antithetic theory of the nature of alternation of generations has been the dominant one; this has profoundly in-

fluenced morphological studies of the Bryophyte sporophyte. In the light of this view the vegetative activities of this structure are minimized with the implication that such activities are undeveloped or incipient. Studies of the development of the sporophyte in living Musci clearly indicate that photosynthesis begins early in ontogeny and continues nearly to the time of capsule ripening. Although the moss apophysis undoubtedly is actively photosynthetic, photosynthesis occurs in the sporophyte long before it is differentiated as well as in those species where the apophysis is poorly developed. The absence of functional stomata in the capsule of Sphagnum palustris does not prevent photosynthesis in that structure. The sporophyte of Andreaea also is already photosynthetic before it emerges from the venter. The same is true of the sporophytes of Funaria flavicans, Bartramia pomiformis, Dicranum sp. and Pogonatum pennsylvanicum. These studies of the sporophytes of representatives of the three groups of Musci, the Sphagnobrya, Andreaeabrya, and Eubrya, along with the author's earlier published studies of the Hepaticean sporophyte, indicate that the sporophyte of the Bryophyta early in ontogeny initiates photosynthetic activity and is thus largely "independent" of the gametophyte in respect to carbohydrate nutrition. These observations are interpreted as supporting the homologous theory of alternation which emphasizes the fundamental similarity between gametophyte and sporophyte.

PHYSIOLOGICAL STUDIES ON MOSSES. I. THE DE-VELOPMENT OF LEAFY GAMETOPHYTES IN LIQUID MEDIA. Samuel L. Meyer, Miller School of Biology, University of Virginia, Charlottesville, Va.—In the literature on the physiology of the Musci, there appear contrasting and indefinite opinions concerning the development of leafy gametophytes from primary protonemata of normally aerial species in liquid media. Results of investigations with Physcomitrium turbinatum (Michx.) Brid. indicate leafy gametophores develop in liquid nutrient media and that neither direct exposure to air nor attachment to a solid substratum is essential for their development. In the experimental species, both the rate of gametophore development and the number of leafy plants formed are less than on a solid nutrient substrate. Gametophytes grown from primary protonemata submerged in a liquid nutrient medium show pronounced morphological modifications when compared to plants grown normally in

Fertilization in Vallisheria. Robert B. Wylie, University of Iowa, Iowa City, Iowa.—The sperms are formed in the pollen grain and retain their integrity as cells until they emerge from the synergid. One of them soon loses its cytoplasm and is carried as a naked nucleus to the polars. Additional evidence is presented in support of the view that the other sperm retains its organization as a cell until it unites with the egg. Male cells with cytoplasm intact were frequently found in contact with the

egg membrane having been killed during the brief interval between their emergence and fertilization. If a second pollen tube enters the embryo sac, its sperms remain as male cells protruding from the other synergid.

A COMPARISON OF EVOLUTIONARY TENDENCIES IN THE FUNGI, CHLOROPHYTA, AND ANIMALIA. Donald P. Rogers, Oregon State College, Corvallis, Ore.—
The three evolutionary series Fungi, Chlorophyta, and Animalia have separately passed through aquatic, amphibious, and terricolous stages. The similar biological problems of such evolution have been differently met by members of the different series. This difference is most striking in the cytological aspects of the life histories: the Fungi ultimately became dikaryotic, the Chlorophyta retained distinct haploid and diploid generations, and the Animalia early became almost completely diploid. The differences in evolution are evidence of the separateness of the series.

ERIE BLACK AS AN ACETO-CARMINE AUXILLIARY STAIN. B. R. Nebel, N. Y. Agr. Exp. Sta., Geneva. N. Y.—In making chromosome counts on plants and plant parts treated with colchicine it was found that in cases where aceto-carmine alone is not satisfactory—as in axillary buds of apple, pear, plum, peach, apricot, and cherry the following method was successful: Hand section center of bud; kill in 3:1 alcohol acetic acid 10 minutes to 24 hours; alcohol 70 three changes; erie black sat. in 70 alcohol 5 to 25 minutes; alcohol 70 three changes; transfer to slide and dissect meristematic parts in aceto-carmine under binocular; cover, heat, squeeze and seal or add Zirkle fluid for permanent mounting. In sporocyte work erie black may also be employed alone or in combination with aceto-carmine if a dark purple nuclear stain is desired.

A More Graphic comparative life-history chart. Dwight M. Moore, University of Arkansas, Fayetteville, Ark.—Charts showing comparatively the usual outstanding stages in the life histories of the representatives of the various phyla have often been presented on radial lines of comcentric circles. This gives no idea of the relative prominence of the various stages. The present chart shows graphically the decrease in the gametophyte in a heart-shaped area bounded by the lines representing fusion of gametes and meiosis, respectively, and the corresponding increase in prominence of the sporophyte generation. Definite colors are used to indicate various structures, generations or other features.

TEACHING AIDS IN BOTANY. I.—THE PLACEMENT TEST. S. M. Dietz and C. J. Gould, Jr., Iowa State College, Ames, Iowa.—The placement test as used in General Botany at Iowa State College has been developed over a period of twelve years. Prior to 1934 it was used in evaluating student ability but since then it has also been used as a means of sectioning students according to their ability and as a basis for modifying the course for groups and individuals. This placement test, consisting of 10-

16 questions and requiring about an hour for administration, is given at the first class contact. The questions are given in pairs and involve a rating of some of the testable, course objectives which include skills and abilities: in reading, observing, recognizing problems, formulating hypotheses, experimenting, interpreting data and drawing logical conclusions. Each year, 650 students are sectioned into 22 high, medium, or low groups. High sections are given more supplementary materials, more freedom in selection of materials and experiments, a wider range of applied problems and more practice in the use of the scientific method. Acquisition of information plays a more important rôle in the low sections. A comparison of rankings of paired questions of the placement test with their corresponding pairs of questions in the comprehensive final examination affords a valuable teaching aid to the individual instructors by: (1) indicating in which type of section an instructor is most capable; (2) indicating the strong as well as the weak points of individual instructors; and (3) affording a quantitative measure of student progress which is used in teacher-conference groups.

ABNORMAL MICROSPOROGENESIS IN TRADESCANTIA. William L. Brown and Edward C. Berry, Henry Shaw School of Botany, Washington University, St. Louis, Mo.-Abnormal microspores and pollen grains, closely resembling types artificially induced in various Angiosperms, have been found to occur under natural conditions in one individual of Tradescantia subaspera Ker-Gawl. Approximately fifteen percent of the microspores of any given anther are abnormal. This number remains fairly constant throughout the flowering period of the plant. Cell walls frequently fail to form in the diads, resulting in microspores with the unreduced number of chromosomes. In such cases delayed cell wall formation by furrowing is initiated in the microspore or pollen stages. Abnormal microspores possess approximately one-third more refractive granules than do normal ones.

THE NATURE OF THE DIVISION OF THE GENERA-TIVE CELL IN TRADESCANTIA AND POLYGONATUM. O. J. Eigsti, University of Oklahoma, Norman, Okla.—The mitotic features involved in the formation of microgametes were studied for Tradescantia occidentalis (Britton) Smyth. and Polygonatum commutatum (R & S) Dietr. In late prophase, the chromosomes of Tradescantia (n-6) are arranged end to end with the longest dimension of the chromosome parallel to the wall of the tube. The metaphasic stage begins when the chromosomes from each extremity migrate toward a central point. During this process the daughter chromosomes separate and subsequently pass into different nuclei. The chromosomes of Polygonatum (n-20) are arranged upon a definite equatorial plate early in the process, from which the daughter chromosomes separate from each other. The addition of colchicine to the culturing medium prevented the migration of prophasic chromosomes in Tradescantia, and inhibited equatorial plate formation in Polygonatum. The formation of nuclei in the pollen tube continued in the presence of colchicine, resulting in the production of a variable number of nuclei. A cytological analysis revealed that each nucleus contains both daughter chromosomes of the doubled metaphasic chromosome. Upon cursory examination the mitotic processes of the generative cell of these plants seemed different in appearance. however, detailed study disclosed a fundamental similarity between the two. Furthermore, the failure of a complete separation of the daughter chromosomes in the presence of colchicine indicates that the mitotic features of the nuclei of pollen tubes are essentially the same as those of an ordinary mitosis. When treated with colchicine, the formation of nuclei without the separation of daughter chromosomes is an interesting feature of cellular behavior in pollen tubes.

Somatic doubling of chromosomes and nodu-LAR INFECTION IN CERTAIN LEGUMINOSAE. Louise Wipf and D. C. Cooper, University of Wisconsin, Madison, Wis.—Cells having the tetraploid number of chromosomes are occasionally present in the root in the root hair region of the following diploid plants: Pisum sativum L., Lathyrus latifolius L., L. odoratus L., Lespedeza tomentosa Siebold, and Vicia villosa Roth. Such cells are located in the parenchyma of the cortex usually near the endodermis and in the region of young secondary roots. There appears to be a definite relationship between the formation of root nodules in pea and vetch and normally occurring tetraploid cells. Wherever nodular stimulation occurs one or more tetraploid cells are found in the proliferating region of the cortex even though the infection thread has not, as yet, penetrated that area. Later the rhizobia occupy only the cells with the doubled chromosome number. Many infection threads cause no stimulation whatever, even though they pass through several layers of inner cortical cells. In such instances no tetraploid cells were observed in the vicinity of the infection threads. The infection thread has a pathogenic effect on the nuclei of the diploid cells which it penetrates; the chromatin linin net work becomes diffuse, the nuclear membrane assumes irregular shapes, and the nucleus takes a much deeper stain than those of adjacent uninfected cells.

MEIOSIS AND THE CULTIVATION OF EXCISED ANTHERS IN NUTRIENT SOLUTION. Walton C. Gregory, The Blandy Experimental Farm, University of Virginia, Boyce, Va.—The cultivation of excised anthers in nutrient media led to abnormal growth and development in Lilium longiflorum and to cessation of growth in Lycopersicum esculentum, "Bonny Best," and in Datura stramonium. This separation of young anthers from the parent plants led to meiotic failure in all three species. Similar reactions in excised buds and in large cuttings indicate that these effects were not caused by the artificial medium. The normal procedure of meiosis in a grafted cutting of Lycopersicum and its failure

in a similar non-grafted cutting indicates an influence on the part of the stock. It is suggested that the meiotic stimulus is not an inherent property of the sporogenous tissue but is furnished to that tissue by more remote portions of the plant.

THE PHYSIOLOGICAL AND GENETIC EFFECTS OF X-RAYS. Karl Sax, Arnold Arboretum, Harvard University, Cambridge, Mass.—The exposure of cells to X-rays produces two types of response. The first is a physiological effect which results in a partial fusion of metaphase chromosomes and a temporary alteration of nuclear development. Nuclei irradiated at prophase or in the resting stage recover from the physiological effect even though many chromosome aberrations are produced. These permanent aberrations are caused by direct X-ray 'hits" and are lethal in diploid cells only when homozygous deficiencies are produced. chromosome aberrations are referred to as genetic effects since they result in deficiencies or position effects. Either the genetic or physiological effect of X-rays may be cell lethal, and presumably both factors are of importance in radiation therapy.

THE USE OF PERICLINAL CHIMERAS IN TRACING THE GERM-LAYER ORIGIN OF TISSUES IN DATURA. Sophia Satina and A. F. Blakeslee, Carnegie Institution of Washington, Cold Spring Harbor, N. Y. -Seed treatment with colchicine is a new and effective method of inducing periclinal chimeras. In Datura the most frequent chimera type had a 4n or 8n epidermis with 2n internal tissue, but types also were found with 2n epidermis and 4n internal tissue. The cortex may be distinguished from the central cylinder as well as from the epidermis by differences in the chromosome number. Such differences may involve chromosome deficiencies as well as doubled chromosome number. Differences in chromosome number and size of affected cells may be used as labels with which to identify the different germ layers from which they have arisen. The evidence seems to indicate that there are at least 3 definite germ layers in Datura. The dermatogen is found to give rise not only to the epidermis but to contribute to internal tissue.

SEED PATTERNS IN LILIUM AND THEIR SIGNIFI-CANCE. A. B. Stout, New York Botanical Garden, New York, N. Y.—The disposition of seeds in capsules of Lilium allows one to construct charts of the relative positions of various classes of seeds in relation to (1) the abortion of ovules, (2) the pollentube behavior in incompatibilities, and (3) the internal stimulations involved in the development of phenospermic seeds. The seed patterns for the fully self-incompatible triploid Lilium tigrinum clone IN-TERMEDIATE show that at least 50 per cent of the ovules are functional when tested in most successful hybridizations. In various individuals and clones of Lilium candidum there is definite evidence that there are two main types of pollen-tube distribution associated with incompatibility; there is (1) a distal distribution in which the pistil and ovary as a whole limit pollen-tube penetration and (2) there is a scattered distribution in which there appears to be selective relations with individual ovules. The distribution of the different sizes of phenospermic seeds indicate that the size of such seeds depends on the distance from well-developed seeds, and that there is the action of stimulating substances, such as growth hormones, which spread through vascular tissues from fertilized ovules and developing embryos.

INHERITANCE OF SEED-COAT COLOR IN PEANUTS. B. B. Higgins, Georgia Experiment Station, Experiment, Ga.—In the course of an extensive breeding project with peanuts, crosses have been made involving some 16 varieties and strains, including varieties with red, flesh, and white seed-coat colors. Generally the inheritance of seed-coat colors followed very closely the genetical segregations previously reported by other workers. However, one variety, Pearl, with a white seed coat differs from any previously reported in that in every cross made the F<sub>1</sub> seed coats are red. In crosses using Pearl with another white variety, Philippine White, which appears from other crosses to have no factor for color, the F<sub>1</sub> seed coats are red and F<sub>2</sub> red, flesh, and white. The results from all crosses made indicate that the Pearl has factors for both red and flesh colors and also two inhibitors both of which are necessary to prevent development of color.

DEVELOPMENTAL PATTERN OF THE LEAF BLADE IN 2N AND 4N ZEA MAYS AS RELATED TO THE STRUC-TURE OF THE GROWING POINT. E. C. Abbe, University of Minnesota, Minneapolis, Minn., L. F. Randolph, Bureau of Plant Industry, U. S. Department of Agriculture, and Cornell University, and J. Einset, Cornell University, Ithaca, N. Y .-In 2n Zea mays, leaf blades 6 to 12 (counting upwards from the coleoptile) are successively and regularly wider for any given length when compared ontogenetically. Measurements of the growing points at the stages when the primordia of these leaves are being formed indicate that the successive growing points also increase regularly in circumference and volume. Increase in cell number is responsible for this increase in size of the growing points, cell size remaining essentially uniform. The leaf blade initial originates as an equatorial bulge at the base of the growing point in corn. The basal circumference of the growing point is therefore taken as a measure of the initial width of the leaf blade. The successively greater circumferences, due to an increase in the number of cells, are directly reflected in the greater relative widths of the associated leaves. In 2n and 4n corn, an ontogenetic comparison of leaf blades 10 shows an analogous condition, the 4n leaf blade being wider than the 2n for any given length. This greater relative width is correlated with a corresponding difference in growing point volume and circumference. The greater volume of the 4n growing point is, however, due to increase in cell size, cell number remaining the same. There is a corresponding difference in nuclear volumes presumably associated with polyploidy.

STUDIES PERTAINING TO THE LIFE HISTORY OF SPECULARIA PERFOLIATA (L.) A. DC., WITH SPECIAL REFERENCE TO CYTOLOGICAL ASPECTS. J. A. Trent. State Teachers College, Pittsburg, Kan .- A comparative study was made of both open and cleistogamous flowers. Megasporogenesis and microsporogenesis were found to be essentially the same in both kinds of flowers. The archesporial cell in the ovule becomes the megaspore mother cell directly. The usual tetrad of megaspores is produced. The chalazal megaspore becomes the functional one, and through its subsequent divisions, a "normal" embryo sac is produced. The antipodal nuclei undergo rapid divisions giving rise to a multicellular "haustorium." The synergids move into the micropyle and give rise to a micropylar "haustorium." Microsporogenesis follows the usual procedure for most angiosperms. The mature pollen grains are 2-3 nucleate by the time they leave the pollen sac. In the open flowers, the pollen grains germinate on the stigma, and the tubes pass to the ovary through the stylar tissue. In the cleistogamous flowers, the pollen grains germinate within the pollen sac. The tubes penetrate the wall of the pollen sac, reach the stigma, and pass to the ovary in the same manner as in the open flowers. The generative nucleus divides within the pollen grain before germination. Fertilization occurs in the usual manner. Endosperm formation may occur in the absence of triple fusion or even the fusion of polar nuclei. The endosperm becomes fully developed in many instances, in both open and closed flowers, in the absence of an embryo.

POLYPLOIDY AND WINTER HARDINESS RELATION-SHIPS IN THE FLOWERING PLANTS WITH REFERENCE TO KARYOGEOGRAPHICAL PROBLEMS. Wray M. Bowden, The Blandy Experimental Farm, University of Virginia, Boyce, Va.-An investigation of the relationships between chromosome number, degree of winter hardiness, and the geographical distribution of the flowering plants was begun by the author in the spring of 1938. To date some 100 species and varieties pertinent to this problem have been examined cytologically. A brief comparison of certain critically significant species indicates that in most cases the degree of winter hardiness is not correlated with chromosome number differences. Furthermore, the evidence indicates that the degree of hardiness depends upon the genetic constitution of the individual. The whole range of variability in degree of hardiness can be found in either diploid species or in polyploid species. The cases reported in the literature which have correlated higher chromosome number with increased altitude or latitude, apparently illustrate only one of the various types of karyogeographical complexes.

A PRELIMINARY INVESTIGATION OF THE ACENA-PHTHENE RESPONSE SEEN IN CERTAIN SEEDLINGS. A. Orville Dahl, Harvard University, Cambridge, Mass.—Hypocotylous swelling, identical with that seen in colchicine-treated seedlings, was noted in Ipomoea L. exposed to acenaphthene (naphthyleneethylene, C<sub>10</sub>H<sub>6</sub>(CH<sub>2</sub>)<sub>2</sub>) fumes. Certain abnormalities in the curvatures of these seedlings suggest that disturbances of morphological polarity are involved. An experimental series utilizing germinating grains of a pure line of Avena sativa L. which received acenaphthene or colchicine treatment has shown marked swelling of the coleoptilar portion of the embryo as well as a general lack of normally polarized growth. Those seedlings receiving acenaphthene treatment differed from the colchicinetreated series in having greater elongation of the coleoptile as well as a peculiar curvature response which directed the tip of the coleoptile away from the scutellum in the direction of the coleorhiza. Cross-sectional views of the coleoptile indicate that with colchicine treatment the swelling noted is definitely greater than that seen with the use of acenaphthene. Preliminary investigation of the histological aspects of these phenomena indicates that matters of cell size and shape in addition to differential maturation of cells are involved. It seems likely that both acenaphthene and colchicine are capable of causing disturbances which bear no direct relationship to their well-known effect upon the spindle.

THE OXYGEN CONSUMPTION OF ISOLATED WOODY TISSUES. Richard H. Goodwin and David R. Goddard, University of Rochester, Rochester, N. Y.— Thin sections of tissues were cut from the trunks of ash and maple trees with a sliding microtome and their oxygen consumption was measured in Fenn micro-respirometers. In trees collected before bud break oxygen consumption is most rapid in the cambial region. Values for the adjacent secondary phloem and xylem are somewhat lower. In the xylem the rate of oxygen consumption becomes progressively lower as one passes toward the center of the tree, reaching a very low but measureable rate in the heartwood. In trees collected after bud break the rate of oxygen consumption of the cambium, phloem, and heartwood is essentially the same as before bud break. In the newly-formed, differentiating xylem, however, it considerably exceeds the cambial rate. The above relations hold true whether oxygen consumption is expressed per gram of fresh weight or per milligram of nitrogen.

A BOTANICAL SURVEY OF BOIS BLANC ISLAND, MAC-KINAC COUNTY, MICH. Marjorie T. Bingham, Cranbrook Institute of Science, Bloomfield Hills, Mich. -Bois Blanc Island, situated in Lake Huron, between Michigan's upper and lower peninsulas is within the Lake Forest vegetation zone. Within its seventy square miles are widely varied vegetation types, with transition belts so limited or entirely lacking that one passes abruptly from one plant community to another. Spring, summer and fall expeditions have revealed nineteen distinctly different communities composed of approximately six hundred species of plants. On the Lake Huron shores one finds not only the expected differences of high, middle and low beach but within a few hundred feet the character of the shore is totally altered. Shores

of the six inland lakes are unlike the Lake Huron shores. The dominant coniferous forest is entirely replaced in certain sections by pure stands of hardwoods. A few old clearings have assumed the aspect of depauperized meadows, others with more moisture are lush with flowers, many are reverting to forest. Fifty per cent of the island consists of nearly impassable cedar swamp. A small area held in reserve by the United States government has remained primeval forest.

SALT MARSH PLANTS IN RELATION TO TIDE LEVELS ON THE CALIFORNIAN COAST. Ira L. Wiggins, Stanford University, Calif.—An accurate determination of the levels at which Spartina leiantha Benth., Distichlis spicata (L.) Greene, Salicornia pacifica Standley, and Frankenia grandifolia C. & S., grow in eight coastal salt marshes from San Diego to San Francisco Bay shows that these species are confined to definite tidal zones. Spartina grows from about mean sea level to somewhat above mean sea high water. Salicornia grows from a short distance below mean high water upward. Distichlis and Frankenia grow on the higher levels of the salt marshes, never dropping below the plane of mean high water. Where the tidal regime is low these two plants approach closely the level of mean high water, Distichlis growing 0.10 feet and Frankenia 0.28 feet above mean high water at a station where that level is 4.00 feet above mean lower low water. Where the tidal regime is greater both grow at somewhat higher levels. A study of the curves of tidal charts suggests that the lower limit to which salt marsh plants can grow is determined by their response to the average total time of submergence during the twenty-four hours rather than to direct responses to the depth of flooding.

Desert flora of northern peru in an exceptionally rainy year. Harvey E. Stork, Carleton College, Northfield, Minn.—The heaviest rainfall that the desert of the Piura Department of Northern Peru had experienced for many years brought out a dense growth of vegetation in 1939. Most of the growth was produced by five species of grasses and ten species of other annuals, seeds of which can remain viable in the sandy soil during long periods of drought. Several perennials depend upon subterranean storage organs to carry them over the dry years, and two woody species survive by sending tap roots to an eight meter depth.

DISTRIBUTION OF BRIGHT BELT TOBACCO ROOTS IN RELATION TO SOIL PROFILE. L. J. Gier, Campbell College, Buies Creek, N. C.—During 1937 and 1938, the root systems of over 700 tobacco plants, from two soils each of the Norfolk and Ruston series of Harnett County, N. C., were examined. The bulk of the root system for each of these plants was in the plowed A horizon, although many feeding roots extended into the A2. Usually only a single root entered the B horizon. Branching seemed to be determined by the moisture content, soil texture, distance from the stem, and cultivation methods.

There was a definite correlation between the total sands, the total colloids, and the root distribution.

DEVELOPMENT OF THE NUT GRASS PLANT (CY-PERUS ROTUNDUS L.). J. R. Jackson and E. V. Smith, Alabama Agric. Exp. Sta., Auburn, Ala .-The nut grass plant consists of tubers and connecting rhizomes, rosettes of foliage leaves, and umbel-bearing scapes. New plant systems develop from isolated tubers by the growth of apical or lateral buds which produce rhizomes terminated either by rosettes of foliage leaves or by tubers arising by secondary growth of the pericycle of the rhizome. A rhizome which terminates in a rosette of foliage leaves later produces a tuber at the base of the leaves. Any new tuber formed in the system either produces rhizomes from terminal or lateral buds or becomes dormant. A system of more than one hundred tubers may be formed in one season. The plant exhibits both collateral and amphivasal bundles, clearly differentiated cortical and stelar regions, secondary growth by the pericycle, and a cork cambium.

A COMPARATIVE STUDY OF THE SUBTERRANEAN MEMBERS OF SEVERAL CROP PLANTS. Howard J. Dittmer, Chicago Teachers College, Chicago, Ill .-Quantitative data were obtained through counts and measurements of the roots and root hairs of Kentucky bluegrass, winter rye, oats, and soy beans. In soil samples 3 inches in diameter and 6 inches deep, bluegrass had far more numerous subterranean members than any of the other plants, and all grasses greatly exceeded soy beans in number, length and surface area. However, only bluegrass exceeded soy beans in volume. Based on these figures the tap root system of soy beans is very poorly adapted for binding soil. The fibrous root systems of bluegrass, winter rye and oats are far more effective, ranking in efficiency in the order given.

METHODS IN AEROBIOLOGY. O. C. Durham, Abbott Laboratories, North Chicago, Ill.—In studying the aerial incidence and dispersal of pollens, fungus spores and insect particles which are recognized as causes of inhalant allergy, wide use has been made of the gravity method of sampling. Consideration is given to the advantages and disadvantages of various devices for securing rapid deposits of aerial debris and for obtaining volumetric counts. The special problems encountered in taking samples from airplanes over land and water are discussed. Attention is given to a practical technic for preparing and counting atmospheric slides.

CHANGES IN GRASSLAND VEGETATION IN WESTERN NORTH DAKOTA 1932 THROUGH 1939. Warren Whitman, Herbert C. Hanson and Roald Peterson, North Dakota Agricultural Experiment Station, Fargo, N. D.—The period of 1932 through 1939 included two seasons of serious drought in western North Dakota. These occurred in 1934 and 1936. The effects of these drought years on the grassland vegetation were greater than the combined effects of any other factors or groups of factors during this period.

The severe seasonal fluctuations in environmental conditions resulted in wide variations in abundance, density, height growth, seed production, and relative proportions of the constituent species of the grassland communities. These changes, however, were not as extensive nor as striking as those reported from the Central and Southern Great Plains.

The most obvious effect of the two seasons of drought on the grassland vegetation was a reduction in basal area and in abundance of the principal species. The losses incurred as a result of the drought have not yet been fully made up, even after three fairly favorable seasons. Bouteloua gracilis, Agropyron smithii, and Stipa comata were all seriously affected, although the latter species made a remarkable recovery as the result of spread from surviving established clumps and from seedlings. Koeleria cristata was reduced by about 80 per cent, and Andropogon scoparius showed a 50 per cent decrease in abundance. Practically all species have shown appreciable recovery since 1937.

Both annual and perennial forbs were reduced in number during the drought years. In most cases, however, large gains were made in the succeeding years. This was especially true of the annual forbs; the perennials showed less fluctuation. Considered over the whole period of observation both perennials and annuals made a net increase. In only a few cases did they become dominant over any considerable extent of native grassland.

Species senescence. Stanley A. Cain, University of Tennessee, Knoxville, Tenn.—The question of species senescence is considered in connection with the problem of limitation, in the East, of epibiotic species to nunnatak areas. That species senescence is a fact is questioned. It is suggested that the apparent lack of aggressiveness of such disjunct and circumscribed occurrences of a species may have a genetical explanation in the isolation of a relatively homozygous marginal portion of the species population as a whole with a resultant narrower ecological amplitude. This phenomenon, coupled with the limited occurrence of suitable edaphic situations in the region of the epibiotics, is sufficient to explain their local occurrence and lack of competitive ability in most situations. It does not seem necessary to hypothesize species "senescence," and the anthropomorphism may easily obscure true ecological explanations.

PLANT ECOLOGY: THE TWO USES OF THE TERM. Frank E. Egler, Syracuse University, Syracuse, N. Y.—American and European "ecology" have developed under an unintentional isolationist policy, with the result that the term ecology now has a double usage, not adequately recognized, and causing unnecessary confusion and criticism. To exemplify this double usage, seven leading textbooks, covering the same subject matter, are summarized in regard to nomenclature: those of Braun-Blanquet, Pavillard, Reynaud-Beauverie, Coulter Barnes & Cowles, Toumey & Korstian, McDougall, and Weaver & Clements. In Europe, as sciences have

expanded and new sciences emerged, it is accepted that the term ecology is etymologically too restricted to embrace the many fields of knowledge emanating from the original ecology of Haeckel; in America the term, regardless of etymology, embraces all fields of botanical knowledge not orthodox morphology, physiology, taxagents and contributes.

ogy, physiology, taxonomy, and genetics.

VEGETATION OF A HIGH MOUNTAIN VALLEY IN SOUTHERN COLORADO. Francis Ramaley, University of Colorado, Boulder, Colo.—The San Luis Valley is about 100 miles long and 40 miles wide. Annual precipitation is only 8 inches but the water-table of the central part is close to the surface. Ecologically, there are (a) floor, (b) margin, (c) rim. The floor bears a greasewood scrub (Sarcobatus) mixed with rabbit brush (Chrysothamnus). The valley margin supports a growth of undershrubs, low species of rabbit brush, snakeweed (Gutierrezie), and sagebrush (Artemisia), with much grama grass (Bouteloua) and some spring-flowering herbs. The valley rim is foothill country with open pinyon-pine woodland and grama grassland, changing to spruce-pine forest at higher elevations. Plant communities besides those already noted include: river-bottom forest, sedge moor, mixed meadow, sandy-soil grassland, oak chaparral, and aspen groves.

Unrecognized initial stage of plant succession and its prominence in soil erosion control in the south-central united states. W. E. Booth, University of Kansas, Lawrence, Kan.—Five members of the Myxophyceae, usually little thought of as being of great positive economic importance, have been found to be of major importance in the Red Plains Region and oak savannah of Kansas, Oklahoma, and Texas. They form a complete, nonerosive layer over hundreds of acres of badly eroded and abandoned crop land. In addition to erosion control this algal growth is valuable in creating an environment favorable to plant succession.

FURTHER STUDIES ON ZONAL STRUCTURE AND GROWTH IN THE SHOOT APEX OF CYCAS REVOLUTA. Adriance S. Foster, University of California, Berkeley, Calif.-Following the seedling stage, the shoot apex increases notably in size and in plants 40-50 years old may reach a diameter of 2.5-3.5 mm. Associated with this increase in bulk of the terminal meristem, numerous, small spirally-arranged foliar primordia arise from the peripheral zone and develop into the alternating "crowns" of cataphylls and foliage leaves. Although the apices of vegetative and reproductive individuals appear to be fundamentally similar in general zonal structure, the form of the apex varies from a blunt cone to a low truncate mound. Apparently these form differences reflect periodic changes in the relative intensity and distribution of growth in certain zones of the apex. In very broad plateau-shaped apices, the rib meristem zone is reduced in area and activity as compared with the very prominent zone of peripheral tissue in which mitoses are frequent. Conversely, in cone-shaped apices, the zone of rib meristem is well-defined and active. The implication of these facts is briefly discussed with reference (1) to the rhythmical growth of the shoot and (2) to the problem of organization and structural evolution of the shoot apex in gymnosperms.

Embryogeny of torreya nucifera. John T. Buchholz, University of Illinois, Urbana, Ill.—Stages from fertilization onward show nearly all stages in the embryogeny. Cleavage polyembryony is the rule for this and probably other species of Torreya. There are 2-4 archegonia of which usually only one is fertilized, though several cases of two adjacent proembryos in separate archegonia were observed. The proembryo, in which walls form after 4 free nuclei, is confined to the lower one-half or two-thirds of the egg. A dormant winter stage follows the formation of from 12-20 cells, with prosuspensor cells only slightly elongated. Morphological and embryological studies support scheme of phylogeny of Taxaceae suggested by Saxton.

A STATISTICAL STUDY OF TWO VARIABLES IN THE SEQUOIAS—POLLEN GRAIN SIZE AND COTYLEDON NUMBERS. John T. Buchholz and Margaret Kaeiser, University of Illinois, Urbana, Ill.—In Sequoiadendron giganteum the diameters of 500 expanded pollen grains gave a mean of  $22.47 \pm 0.05$  mu ( $\sigma = 1.47$ ). In Sequoia sempervirens an equal number of pollen grains gave a mean diameter of  $32.85 \pm 0.11$  mu ( $\sigma = 2.60$ ). While the extremes overlap slightly, there is no question as to the significance of this difference which is 85.7 times the standard error. The cotyledon numbers were similarly studied in several hundred seeds of these two forms and the results show that the Big Tree has a mean of 3.7 and the Redwood 2.1.

THE EMBRYOGENIES OF BIOTA AND THUJA. Phyllis L. Cook, Ardmore, Pa.—Biota and Thuja repressent two types of embryogeny, the former exhibiting extreme cleavage polyembryony, and the latter, simple polyembryony with only traces of cleavage. The proembryos are similar except for size. Certain of the proembryonic cells assume the rôle of prosuspensors, while the three to five most distal cells contribute the embryos. In Biota each one of these distal cells gives rise to one embryo, while in Thuja, all three to five contribute to one embryonic body. In Biota, each embryonic cell divides, the distal cell remaining embryonic and the proximal becoming the primary suspensor cell. In Thuja, no primary suspensor is formed. Embryonic competition is keen in Biota, entailing great elongation and twisting of suspensors. Several embryos become massive and form secondary suspensors. In Thuja, competition is limited to the number of zygotes, and secondary suspensor formation begins early. After the embryos become massive, tissue development is similar in the two genera, except for the greater size of all structures in Biota.

DEVELOPMENT OF THE FEMALE GAMETOPHYTE AND EMBRYO IN REGNELLIDIUM DIPHYLLUM. Noe L. Higinbotham, Columbia University, New York,

N. Y.—The cell by cell formation of the female gametophyte from the megaspore as well as the development of the fertilized egg into a young plant has been observed in the monotypic genus Regnellidium. As in the related Marsilea and Pilularia, the archegonium is formed at the spore apex, but in Reqnellidium it is enclosed by the folds of the apical papilla. Fertilization is accomplished in about 25 hours. As described for Marsilea and Pilularia, the first division is parallel with the long axis of the archegonium. The following divisions in Regnellidium, however, do not agree with the descriptions given for those other genera. The second division. in the best cases found, is not transverse but is again parallel with the long axis of the archegonium and divides the embryo unequally into four cells. The third division then follows transversely to the archegonial axis. Eventually a cotyledon with a distinct blade is developed and later leaves gradually attain the mature bipinnate form. Regnellidium closely resembles Marsilea and Pilularia, and the bearing of the present data on the relationships within the family is discussed.

THE DEVELOPMENT OF THE EMBRYO OF BARLEY. James Merry, University of Michigan, Ann Arbor, Mich.—The embryonic development of barley was studied in normal embryos and in others excised and grown on nutrient agar. Those excised and cultured showed no further development such as would take place in the ovary, but grew into small plants which fixed the particular stage of the embryo at the time of culturing. A series of such plants from embryos of various ages was studied and the relationships between the parts of the embryo determined. The scutellum and coleoptile are differentiated previous to and separately from the stem axis and are thus considered to be structures peculiar to the embryo and in no way comparable to the lateral organs of the stem.

RELATIVE GROWTH OF DIFFERENT RADII IN CROSS-SECTIONS OF DEVELOPING OVARIES OF IRIS FULVA AND I. HEXAGONA VAR. GIGANTICAERULEA. Herbert P. Riley, University of Washington, Seattle, Wash. In ovaries of giganticaerulea the shortest radius (the septum between carpels) grows faster relative to the radius through the midrib of the carpel than in fulva. The relative growth rates of midrib-radius and longest radius (about 30° from the midribradius) are about the same in both species until the capsule is partially formed at which time in fulva the former grows somewhat faster relative to the latter. The rates of midrib-radius in relation to the radius half-way between the longest and shortest radii (about 45° from the midrib radius) is the same in both species. The 45° radius grows faster relative to the septum in giganticaerulea until about anthesis, when it slows down in that species and accelerates in fulva; these radii are about the same in length in the largest capsules of both species. There is an absolute increase in the size of parenchyma cells of the ovary wall during development.

Final shape depends on the original shape when the ovary is first organized and the relative rates of development of the different radii.

FACTORS RESPONSIBLE FOR THE DEVELOPMENT AND DISTRIBUTION OF THE ENDODERMIS IN MONOCOTYLE-DONOUS PLANTS. D. S. VanFleet, Indiana University, Bloomington, Ind.—Investigations on the formation, distribution and maturation of the endodermis in monocotyledonous plants indicate the presence of an oxidase system associated with endodermal tissue. The extracellular and lamellar location of the oxidases is a factor in the location and ultimate development of the endodermis. All of the characteristic configurations of the endodermal cell. including the Casparian strip and the centripetal thickening associated with many mature cells; is believed to be an expression of a differential in oxidase and antioxidase distribution and activity. The presence of the endodermis in underground stems and its absence in most aerial stems may thus be accounted for. Atmospheric oxygen or organic peroxides in the cortex and enzymatic effusa from the phloem result in an endodermis or mestome sheath whose configuration varies with environmental, cortical and stelar conditions. These conclusions were reached as a result of the microchemical examination or experimental modification of environmental conditions in 86 species distributed through 62 genera and 14 families.

THE OCCURRENCE OF VESSELS IN THE MONOCOT-YLEDONEAE. Vernon I. Cheadle, Rhode Island State College, Kingston, R. I.—All parts of the plants of over 100 species of 75 genera in 18 families were investigated by use of both sections and macerations of the tissues. Vessels were found in the roots of all species investigated. Vessels with only porous perforation plates predominate in only the Agavaceae. Vessels with only scalariform perforation plates predominate in the Amaryllidaceae, Liliaceae, Araceae, Pandanaceae, Bromeliaceae, Strelitziaceae, Trilliaceae, Typhaceae, Zingiberaceae and are found in four species of the Agavaceae. Vessels are found in the stems of a comparatively small number of the species and where found generally have scalariform perforation plates. They are present in the Smilacaceae, Pandanaceae, Palmae, Gramineae and in seven species of the Liliaceae. In Acorus and Monstera there may be a few vessels. Vessels are found in the leaves of even fewer species. They have been noted only in the Gramineae, Palmae, Pandanaceae, Smilacaceae and in Cordyline terminalis and Dracaena fragrans. It is obvious that, aside from the roots, the organs of the plants in the Monocotyledoneae are more often characterized by the absence of vessels rather than by their presence. Futhermore, in the majority of cases, the vessels generally have the more primitive types of perforation plates.

SEPTAL GLANDS IN THE LILIALES. Carrolle E. Anderson, Adelphi College, Garden City, Long Island, N. Y.—The structure of the septal glands was studied in the pistils of two hundred and fifteen

species representing ninety-four genera of the Liliales. These septal glands are intercarpellary spaces lined with a simple or a several-layered glandular epithelium. Glands are not present in all genera and species, but the Juncaceae is the only family studied in which they are completely lacking. The size of the gland varies greatly both as to radial and vertical extent. The position of the glands in respect to the locules varies also, some glands being found only below the locules (basal); some appearing at about the middle of the ovary (median); and some visible only at the top of the ovary (terminal). External secretory pores may be present or absent. Secretions from the glands may be poured out directly through basal pores or grooves; they may flow down from the summit of the ovary through tiny tubes; or they may be secreted through terminal or median pores and then flow down intercarpellary grooves to the base of the floral organs.

THE BORDER-PARENCHYMA AND THE VEIN-RIBS OF CERTAIN DICOTYLEDONOUS LEAVES. Richard R. Armacost, University of Iowa, Iowa City, Iowa.—A survey of seventy species, involving mesophytic leaves from woody and herbaceous plants as well as broad-leaved evergreens, showed that the borderparenchyma invested all minor veins as an unbroken sheath one layer of cells in thickness. Its presence greatly increased the area for mesophyll contacts with the conductive system (from 122 per cent to 238 per cent), while its volume was from 300 per cent to 1100 per cent greater than that of the enclosed vein. Dorsi-ventral extensions (vein-ribs) from the border-parenchyma to the epidermis were present in all these leaves. Experiments showed that these vein-ribs promptly conducted eosin solution to the epidermis from veins of certain categories in every species tested. Evidence indicates that the border-parenchyma and the vein-ribs play an important part in conduction between blade tissues and veins.

THE RELATION BETWEEN PLANE OF CELL DIVISION AND ORGAN FORM IN CUCURBIT FRUITS. Edmund W. Sinnott, Columbia University, New York, N. Y .-Differences in fruit shape in cucurbits are due to differences in growth rate between various dimensions, either during early development or throughout growth. In a number of races differing in dimensional growth rates, the angle between the polar axis of a dividing cell and the longitudinal axis of the ovary was measured for a large number of cells. Frequency of division in a given plane was found to be closely related to growth rate of the ovary in that plane. Whatever determines the plane in which a cell divides is therefore concerned with the control of form differences in the developing organ. Plane of division was found to be most variable in metaphase and least in telophase, indicating that the position of the figure is not established at the beginning of mitosis.

THE PEG OF THE CUCURBITS: A STRUCTURE IN-DUCED BY GROWTH HORMONE? Carl D. LaRue, University of Michigan, Ann Arbor, Mich.—Squash seeds with outer coats removed, germinated horizontally, form pegs on the lower side, but upright, exposed to light from one direction, produce pegs on the shaded side. Upright in darkness they develop pegs entirely around the hypocotyls. If the inner coats are completely removed, pegs are not formed, but if coats are left intact on one side pegs appear on that side. Seeds without inner coats but smeared on one side with 0.1% 3-indole acetic acid in lanolin form pegs on the lanolin side. No transfer of hormone from seed coat to embryo appears after 24 hours germination, but after 48 hours germination such transfer is indicated by tests such as those shown above. Avena tests show a decrease of hormone in inner coats and an increase of hormone in embryos after 48 hours germination. Similar results were obtained with pumpkin and watermelon seeds. It appears that the embryos contain enough hormone for normal growth but require transfer of an additional amount from the inner coat to produce pegs. The peg appears to be the only plant structure known which depends on hormones for its existence.

CELL NUMBER IN THE AVENA COLEOPTILE. G. S. Avery, Jr., H. B. Creighton, Connecticut College, New London, Conn.—In order to better understand the activity of hormones, enzymes, etc. in living tissues, and to evaluate in precise terms any data which may be obtained, the number of cells participating in the reactions must be known. Avena coleoptiles of different lengths (1.5, 4, 10 mm., etc.) were cut into  $100/\mu$  segments. The total number of cells was determined in each successive segment. As the coleoptile grows longer, the total number of cells decreases in each segment, except at the distal end; the longer the coleoptile the fewer the cells in the basal segments. In coleoptiles of all lengths the number of cells in the tip segment is about the same, and the number of cells increases in successive segments from tip to base.

FORM CHANGES IN THE APICAL MERISTEM EURING GROWTH. W. Gordon Whaley, Barnard College, Co-

lumbia University, New York, N. Y.—The shape of the shoot apical meristem of Lycopersicon was studied in three dimensions by the construction of models from serial longitudinal sections. The actual meristematic mass was found to have a roughly spiral shape, the form of the spiral changing as the plant matures. Leaf primordia are formed at the lower, differentiating end of the spiraling meristematic mass.

GROWTH AND DIFFERENTIATION OF CORN PLANTS IN RELATION TO NITROGEN SUPPLY. Ilda McVeigh and Paul R. Burkholder, University of Missouri, Columbia, Mo.—Several inbred lines of corn and their hybrids were grown for 6 weeks in four series of nutrient sand cultures with nitrogen (NH4NO3) varied as follows: 0.2, 0.8, 1.6, 3.2, and 9.6 millimoles per liter of solution. Hybrid vigor, as indicated by fresh and dry weights, varied directly with the nitrogen supply, and to a greater or less degree among the different crosses. Thin sections were prepared for observations on meristems, parenchyma and vascular bundles. In plants grown on limited nitrogen, phloem was poorly differentiated and the pitted xylem elements were small, thin-walled, and filled with protoplasm. In the same lines and crosses, cut at the same age, but grown on high nitrogen, the sieve tubes and companion cells were well differentiated and the pitted xylem vessels were large, thick-walled, and without protoplasm. Diameter of the internodes in all plants varied directly with size of parenchyma cells and amount of supplied nitrogen. Development of terminal meristems was arrested in plants grown on deficient nitrogen. With increased nitrogen, terminal inflorescences developed at different rates in different lines, and more rapidly in heterotic crosses between inbreds than in the inbreds themselves. Heterosis is here related to the ability to use nitrogen. Increase in body size with increasing nitrogen supply is dependent upon the specific growth and differentiation of cells in the different tissues.

### PALEOBOTANICAL SECTION

ABSTRACTS OF THE PAPERS PRESENTED BEFORE THE PALEOBOTANICAL SECTION OF THE BOTANICAL SOCIETY OF AMERICA, COLUMBUS, OHIO, DECEMBER 28 TO 30, 1939

A NEW CUPULE FROM THE LOWER CARBONIFEROUS OF SCOTLAND WITH A NOTE ON THE MORPHOLOGICAL SIGNIFICANCE OF PALAEOZOIC CUPULES. Henry N. Andrews, Jr., Washington University, St. Louis, Mo.—A cupule from the Lower Carboniferous oil shales of Scotland is described as a new genus, the type species of which is Megatheca Thomasi, named in honor of Dr. H. H. Thomas. The cupule is an extraordinarily large (6.2 cm. long) tulip-shaped compression composed of 6 lobes free for twothirds of their length. Prominent surface reticulations are held to represent the original presence of a "Dictyoxylon" cortex. The fossil was found associated with Telangium affine (L. & H.). The hypothesis is put forward that the cupular lobes represent modified (originally sterile) telomes enclosing a group, or groups, of fertile telomes. The latter according to Miss Benson's theory became specialized to form the integument and its enclosed seed.

Additional notes on rotodontiospermum, a medullosan seed. Waldo E. Steidtmann, Bowling Green State University, Bowling Green, Ohio.— Newly discovered material of Rotodontiospermum illinoiense, a medullosan seed from the Pennsylvanian of Illinois, illustrates the presence of a well defined micropyle and slerotestal modifications not observed in the original material.

MALE FRUCTIFICATIONS OF THE MEDULLOSACEAE AS ILLUSTRATED BY TWO NEW SPECIES OF DOLEROTH-ECA. James M. Schopf, Illinois State Geological Survey, Urbana, Ill.-In addition to providing abundant material of Medullosa, aff. M. Noéi, Rotodontiospermum, and Alethopteris, aff. A. serli, coal balls from the Calhoun horizon (upper McLeansboro) have afforded a wealth of material of Dolerotheca. Their morphology and histology is discussed and evidence presented favoring their classification in the Medullosaceae. At the same time the specific uncertainties of their attribution are emphasized which render it unlikely that biological correlation can ever be sufficiently well established to permit taxonomic merging of any of these genera. It is suggested that Heterangium and Heterotheca from the lower Carboniferous are the best examples of pre-medullosan ancestry now known. Male spores of Heterotheca, Telangium and Crossotheca are characterized as "Filicisporic"; those of the medullosan alliance as "Dolerosporic." These spore distinctions may be exceedingly useful in outlining the geologic distribution of two major pteridosperm divisions.

GROSS ANATOMICAL VARIATIONS IN MEDULLOSA, AFF. M. NOÉI. Waldo E. Steidtmann, Bowling Green State University, Bowling Green, Ohio, and James M. Schopf, Illinois State Geological Survey, Ur-

bana, Ill.—The gross anatomy of Medullosa stems, aff. M. Noéi, from the higher McLeansboro Calhoun coal horizon is presented by a series of diagrams. Stelar configurations show considerable variation but most striking of all characters is the distribution of internal periderm. The characteristics of the periderm possibly provide the best available means of judging ontogenetic age of any particular specimen. For taxonomic purposes, the characteristics shown by the primary wood appear to be of considerable significance.

PROGRESS REPORT ON INVESTIGATIONS OF OHIO PSARONII. A. H. Blickle, University of Cincinnati, Cincinnati, Ohio.—Numerous specimens of silicified Psaronius stems have been obtained from beds of Conemaugh and Monongahela ages in Ohio. A number of new species are described and compared with stems previously identified. The status of the present investigation of anatomical and morphological features of the material is reviewed with reference to taxonomic interpretation.

COAL FLORA STUDIES: LEPIDODENDRALES. Fredda D. Reed, Mount Holyoke College, South Hadley, Mass.—Among the many and diverse plant tissues found in coal balls from Harrisburg, Ill., were fragments, both vegetative and reproductive, with ample structural preservation to establish their position in the order Lepidodendrales. Of these fragments there were six species of leaves (Lepidophyllum); rhizome and rootlets of Stigmaria; cone axis, cone scale, microsporangium containing microspores, isolated megaspores of Lepidostrobus; and developmental stages of Lepidocarpon. While it is possible that some of the vegetative and reproductive genera might be related and reduced to the same plant, yet the variation within a genus, as in Lepidophyllum, and the new species reported testify to the variability and complexity of the order.

MAZOCARPON FROM THE HIGHER MC LEANSBORD BEDS OF ILLINOIS. James M. Schopf, Illinois State Geological Survey, Urbana, Ill.—The morphology of well preserved and abundant male and female cones of Mazocarpon is presented. The mega- and micro-spores are characterized and features of the female gametophyte illustrated.

Evidence is presented for favoring a sigillarian alliance for *Mazocarpon* on a somewhat more substantial basis than previously.

With respect to biological considerations it is concluded that *Mazocarpon* has no close affinity with any of the Paleozoic seed-bearing lycopods and retains the essentials of the free-sporing habit. The megasporangium is highly developed to a state incomparable among either the fossil or modern representatives of Lycopsid plants.

A NEW SPECIES OF CORDAITES FROM THE PENNSYL-VANIAN STRATA OF IOWA. L. R. Wilson and A. Johnston, Coe College, Cedar Rapids, Iowa.—Coal ball studies in Iowa have produced many petrifactions, among which are roots, stems, and leaves of Cordaites. An anatomical study of the stems show that they are undoubtedly of a new species and most closely related to Cordaites michiganensis Arnold. The name C. iowensis is proposed for the species.

PROTOLEPIDODENDRON, WITH SPECIAL REFERENCE TO THE UPPER DEVONIAN OCCURRENCE IN SOUTHEASTERN NEW YORK. Harlan P. Banks, Cornell University, Ithaca, New York.—The essential characters of the genus Protolepidodendron are reviewed and its separation from the numerous other early lepidodendrid genera discussed. New species from New York deposits are described and illustrative material exhibited.

SOME RELATIONS BETWEEN PALEOBOTANY AND EVOLUTIONARY THEORY (SUMMARY). Edward C. Jeffrey, Harvard University, Cambridge, Mass .- In recent years the experimental side of the biological sciences has made great progress, a situation which is entirely advantageous. As a consequence the fundamental fact that biology is to a very large degree an historical science is sometimes lost to view. One consequence of this tendency has been the diminished regard for the biogenetic law or the hypothesis of recapitulation. It has been, for instance, objected that the gill arches of the mammalian embryo are not a persistence of structures derived from a fish-like ancestry, but on the contrary represent a physiological adaptation for the respiration of the surrounding amniotic fluid. This criticism loses sight of the unquestionable physiological fact that foetal respiration in the placental mammals is carried on through the blood vessels of the placenta. Further, the young mammal has fish-like segmented musculature. Even the rashest experimentalist could scarcely urge that this corresponds to the necessity for swimming about in the maternal amniotic fluid. Even if the criticisms of the recapitulationary hypothesis outlined above are frivolous and inaccurate, there may well remain a doubt on account of the extreme interval between fishes and mammals.

In the case of the Conifers we have a continuous series from the Paleozoic to the present, which comprises not only external form but important details of internal organization. One relatively ancient group of Conifers is the Araucarians, now confined to the southern hemisphere but once prevalent throughout the world. The Cretaceous Araucarians closely resembled living forms, but presented certain peculiarities, which make impossible the conventional view that they are nearly related to the Paleozoic group, the Cordaitales. It is an interesting fact that the significant characters of later Mesozoic Araucarians are represented in a recapitulationary form in living representatives of the group. As a consequence of this situation, which has many counterparts in the coniferous series, we have to do with the actual persistence of ancestral characters in the young of closely related modern forms. To employ the words of a forceful President of the United States, we have to do with a condition and not a theory, particularly as no flight of physiological imagination can correlate the ancestral structures persisting in modern Araucarians with any physiological utility. The biogenetic law thus received new and very strong support from the side of fossil Conifers.

Another undesirable tendency is to advance theories of relationship or at least of resemblance based on a single character. This situation is well represented by the form genera of Kraus, in which a single characteristic figures as a determinant. Thus we have the terms Araucarioxylon, Cupressinoxylon, Pityoxylon, etc., based on single characters. The absurdity of the classification even from the standpoint of the mere species maker is clear when it is pointed out that all surviving groups of Conifers, except the Abietineae, once had the ligneous characteristics of the Cupressinoxylon type. Further, until recently none of the true Araucarian fossil woods has found a place under the form genus Araucarioxylon. The well known Whitby jet, for example, has been referred to Araucarioxylon under the specific title of A. Lindleyi. Jet as a matter of fact belongs on the basis of recent comparative studies to a group very remotely related to Araucarian conifers. Conversely many woods of true Araucarian affinities beyond question have been referred to Cupressinoxylon. It has been recently proposed to refer all fossil woods to the genera of Kraus. In the words of another forceful President of these United States, this would be going back to the horse and buggy days!

It can not be too strongly emphasized that the identification of fossil remains can only be satisfactorily carried out on the basis of comparative anatomical characters and can not rest on the single features adopted in the system of Kraus or similar classifications based on characters of organization. It may further be added that the evolutionary aspects of wood, than which there is no more continuous or important biological document, can best be studied in the light of the biogenetic law.

The origin of the pteridophytes. Gilbert M. Smith, Stanford University, Calif.—The theory of a direct origin of pteridophytes from algae is discussed. Green algae are shown to be the only potential ancestors and reasons are given for thinking it very improbable that they evolved directly into pteridophytes. Bryophytes and pteridophytes are considered phylogenetically related because of the many common features. The possibility of their derivation from a common ancestor is discussed and then rejected. An origin of pteridophytes from an anthocerotean type of bryophyte is considered the most probable because of the many similarities in sporophytic and gametophytic generations of the two.

LATE TERTIARY FLORAS FROM THE GREAT BASIN AND ADJACENT AREAS: THEIR CLIMATIC, FLORISTIC AND STRATIGRAPHIC SIGNIFICANCE. Daniel I. Axelrod, United States National Museum.—An arid north Mexican flora ranged from southeastern California into northern Mexico during Middle Miocene time, when the redwood forest flora lived in the northern Great Basin and Columbia Plateau. Late Miocene and early Pliocene elevation of the Sierra Nevada-Cascade barrier along the western edge of the province resulted in a lowered rainfall and greater extremes of temperature at the north, allowing the northward migration of many members of the southern element.

Late Tertiary floras distributed from southern Oregon and Idaho southward for 900 miles into the Mojave and Colorado Deserts, show that the members of this group are represented in the three generalized communities which may be distinguished over the area at this time—a montane forest at the north, a central woodland association and a desert-border community at the south.

The nature of the climate over the region is discussed briefly, later Tertiary floristic changes over the region are indicated, and a basis for correlation is presented.

Adolph Carl Noé. Fredda D. Reed, Mount Holyoke College, South Hadley, Mass.

# PHYSIOLOGICAL SECTION

ABSTRACTS OF THE PAPERS PRESENTED BEFORE THE PHYSIOLOGICAL SECTION OF THE BOTANICAL SOCIETY OF AMERICA, COLUMBUS, OHIO, DECEMBER 28 TO 30, 1939

THE TOXICITY OF SULFANILAMIDE IN TOBACCO. Ernest L. Spencer, The Rockefeller Institute for Medical Research, Princeton, N. J.-Sulfanilamide in low concentrations produced marked symptoms of toxicity in Turkish tobacco plants (Nicotiana tabacum L.) grown in sand or water cultures. The first symptom is an interveinal chlorosis near the tips of the youngest leaves. As this becomes more severe, terminal growth is retarded but no necrosis appears. Severe cases are marked by the appearance of many strap-shaped leaves. If the chemical is withheld, plants may outgrow the disease soon after its onset. This type of toxicity is apparently dependent on the structure of the sulfanilamide molecule as a whole, for closely related compounds, such as sulfanilic acid, benzenesulfonamide, p-amino-benzamide, aniline and p-toluene sulfonic acid produced no symptoms resembling those described. Sulfapyridine, which contains the sulfanilamide structure, produced symptoms similar to those described above. A striking similarity was noted between the symptoms of sulfanilamide toxicity and those characteristic of frenching, a physiological disease of tobacco. These observations suggest that frenching of tobacco may possibly be due to the formation, in the plant or in the soil, of an organic toxin similar in structure to sulfanilamide.

THE MINERAL COMPOSITION OF YEAST ASH FROM SPECTROSCOPIC ANALYSIS. Oscar W. Richards and Mary C. Troutman, Research Department, Spencer Lens Co., Buffalo, N. Y .- A pure strain of Saccharomyces cerevisiae was grown in Williams' medium, in Williams' medium enriched, and in malt extract medium. After washing, the yeast was ashed in platinum. Spectroscopic plates were made of ashes of the yeast, of the components of the medium, of autolyzed yeast and yeast extract to determine which elements were present. The following elements were found present in the yeast ash: Ba, Bi, B, Ca, Cr, Cu, Au, Fe, La, Pb, Mg, Mn, P, Pt, K, Ag, Na, Tl, Sn, and Zn. Autolyzed yeast contained Al in addition. Most of the trace elements were present in the asparagine, and the large number of impurities present may be a more important reason for its nutrient value for micro-organisms than its nitrogen content. The importance and origin of the trace elements will be discussed.

GLYCOSIDE FORMATION IN PLANTS FROM ABSORBED ETHYLENE CHLOROHYDRIN, o-chlorophenol and chloral hydrate. Lawrence P. Miller, Boyce Thompson Institute for Plant Research, Inc., Yonkers, N. Y.—In a previous report it was shown that potato tubers and gladiolus corms form  $\beta$ -(2-chloroethyl)-d-glucoside from absorbed ethylene chlorohydrin. Experiments with other plant tissues and with other chemicals have shown that the formation

of glycosides in plants from nonnaturally occurring substances can take place quite generally among the higher plants. A crystalline acetyl derivative of a chlorine-containing glycoside formed in tomato plants grown in sand cultures with chloral hydrate added to the nutrient medium has been prepared and the acetyl derivative of the glycoside formed in gladiolus corms from absorbed o-chlorophenol has also been obtained in crystalline form. Analysis of the latter and comparison with authentic  $\beta$ -o-chlorophenol-d-glucoside tetraacetate showed that, contrary to what might have been expected, the glycoside in question is not  $\beta$ -o-chlorophenol-d-glucoside but a glycoside not yet identified. Similarly, the chlorinecontaining glycoside which has been obtained from tomato plants treated with ethylene chlorohydrin is not identical with that formed by ethylene-chlorohydrin-treated potato tubers or gladiolus corms.

A STUDY OF CELLULAR CHANGES AND DRY MATTER CONTENT IN THE GROWING REGION OF THE PRIMARY ROOT OF COWPEA SEEDLINGS. Mary E. Reid, National Institute of Health, Washington, D. C .- The growing region of the primary roots of young cowpea seedlings was cut into successive segments measuring 1, 2, 2 and 5 millimeters in length, beginning at the tip. Some of this material was used for dry weight determinations of the different regions, and similar material was preserved and sectioned for microscopic study of nuclear and cellular activity, the number and sizes of cells and thickness of walls. The relative sizes of cells in the above segments averaged 1, 3, 14, and 30, and the dry matter 23, 16, 8.4, and 6.2 per cent, respectively. Whereas, a volume increase of 1,300 per cent occurred during the transition of a cell from the condition in the embryonic region to that in the region of elongation (third segment), the dry matter increase was 400 per cent

RELATION OF VITAMIN C TO CELL SIZE IN THE GROWING REGION OF THE PRIMARY ROOT OF COWPEA SEEDLINGS. Mary E. Reid, National Institute of Health, Washington, D. C .- Determinations were made of the indophenol-reducing value (vitamin C) of segments of primary roots of cowpea seedlings similar to those described above. A decreasing gradient extending from the embryonic zone to the region of maturation was characteristic of the vitamin C values obtained per unit of fresh weight, whereas the content of the vitamin per cell formed a gradient in the reverse direction. A gain of over 700 per cent in vitamin C per cell occurred during its transition from the meristematic stage in segment 1 to the phase of elongation found in segment 3. The relative amount of increase in volume of the cell was more than twice that of the vitamin. The increase in dry matter per cell more nearly paralleled that of the vitamin.

ANATOMY OF THE STARCH GRAIN. Sophia H. Eckerson, Boyce Thompson Institute for Plant Research, Inc., Yonkers, N. Y .- The method of study used is that of slow disintegration and watching in the microscope as the structural features are uncovered. A very satisfactory procedure is: treatment of the grains with 7.5 per cent hydrochloric acid for 10 to 30 days (Hanson and Katz) followed by calcium chloride or nitrate on the slide. On the outer part of the grain is a thin membrane-like envelope, elastic and pliable in the young grain from living cells; thicker, hard and brittle on the dry mature grain. Against the inner surface of this envelope is a layer consisting of narrow, concentric bands cemented together by a colloidal substance. In the inner part of the grain is a system of radial strands, spreading from the growth center to the inner surface of the concentric band layer. The concentric bands and the radial strands are made up of microspherites (Wieler)—tiny, globoid, birefringent units, held together in rows by and covered with a viscous, colloidal substance. Photographs have been made of all these structures, both in their natural position in the grain and isolated from the grain.

A DIFFERENTIAL VOLUMETER FOR MICRO-RESPIRA-TION MEASUREMENTS. Kenneth V. Thimann and Barry Commoner, Harvard University, Cambridge, Mass.—A micro-respirometer of differential volumeter type is described. By making the experimental vessel small, the control vessel large, and the capillary very fine, high sensitivity is obtained, while the use of a relatively large surface of KOH allows rapid CO<sub>2</sub> absorption. Solutions can be added quantitatively without disturbing the measurements. A drop movement of 1 mm., which is easily measured, indicates a volume change of about 0.06 mm<sup>3</sup>. A double water-bath and a shaking device with instantaneous release are used. The apparatus enables readings to be made at 1-minute intervals on a single 3 mm. section of Avena coleoptile.

EXPERIMENTS ON THE RELATION BETWEEN RESPIRATION, PROTOPLASM STREAMING AND GROWTH IN THE AVENA COLEOPTILE. Kenneth V. Thimann and Barry Commoner, Harvard University, Cambridge, Mass.—Using the vessel described above, it has been found that auxin, in concentrations which accelerate protoplasmic streaming, produces very little, if any, increase in respiration in coleoptile sections, whether with or without sugar. The specific effects of a number of respiratory inhibitors on growth and on respiration have been studied.

OXYGEN INTAKE AND CARBON DIOXIDE OUTPUT OF DORMANT GLADIOLUS BULBS. Norwood C. Thornton and F. E. Denny, Boyce Thompson Institute for Plant Research, Inc., Yonkers, N. Y.—Gladiolus bulbs, held dormant by storage in moist soil at room temperature, when removed from the soil, show, at first, a very low rate of CO<sub>2</sub> output, which then increases rapidly until a maximum is reached after 24 to 48 hours. Then there is a gradual return to the original low rate. The rapid output of CO<sub>2</sub> in the early stage is not due merely to a release of CO<sub>2</sub>

previously absorbed in the tissue, since  $O_2$  absorption is found to occur concurrently with  $CO_2$  output. Indeed, for the first 10 to 20 hours following the removal of the bulbs from the soil and preceding the rapid increase in the rate of production of  $CO_2$ , the volume of the  $O_2$  absorbed is greater than that of the  $CO_2$  released. Finally, there follows, for as long as 14 days, an equal rate of uptake of  $O_2$  and output of  $CO_2$ . During these tests the bulbs are not exposed to change in temperature, and they remain dormant when planted again in soil.

RESPIRATION AND OXYGEN REQUIREMENTS FOR GROWTH OF NUPHAR ADVENUM AND OTHER WATER PLANTS. Harlow E. Laing, University of Michigan. Ann Arbor, Mich .- Studies of the respiration and oxygen requirements for growth were made with Nuphar advenum, Typha latifolia, Peltandra virginica, Pontederia cordata, Nymphaea tuberosa, Acorus Calamus, Sagittaria latifolia, Sparganium eurycarpum, Asclepias incarnata, and Scirpus validus. A modified Pettenkofer method was used. Moist gaseous mixtures of air, 10, 4.6, 3.0, 1.5, 1.0, 0.4, and 0.1 per cent of oxygen and purified nitrogen were used as media. In some experiments the plant material was immersed in water through which either air or purified nitrogen gas was bubbled. In all species, the rhizomes or corms were able to respire anaerobically for unusually long periods of time without any noticeable injury. The oxygen requirements for growth were found to vary with the species, the optimum being below one per cent of oxygen for Nuphar advenum, 1 to 3 per cent for Peltandra virginica, and approximately 4.6 per cent for Typha latifolia, while Pontederia cordata grew almost equally well in concentrations of oxygen varying from about one per cent to 10 per cent. Maximum root growth occurred in moist air regardless of the species. Observations were made on the effects of different concentrations of oxygen on the leaves of certain species.

HYDROGEN ASSIMILATION IN GREEN PLANTS. H. Gaffron, University of Chicago, Chicago, Ill.— Oxygen and carbon dioxide are the only gases known to participate in large amounts in the metabolism of green plants. Now it has been found that algae of the family Scenedesmus can activate and utilize molecular hydrogen which normally is an inert gas for all plants. When incubated in hydrogen for a few hours in the dark, the algae will begin to absorb hydrogen in connection with several metabolic processes. They reduce metabolites as well as molecular oxygen provided the latter is given in small concentration. Even more important seems the reduction of carbon dioxide in the light with the simultaneous absorption of twice the volume of hydrogen. The oxyhydrogen reaction in the dark and the hydrogen photosynthesis are distinct and separate reactions because the photochemical process is by far the faster one. The rate of carbon dioxide reduction with hydrogen is proportional to the light intensity, but to a certain degree only. If the light intensity becomes too high, the absorption of hydrogen stops and the algae return to normal photosynthesis with the liberation of oxygen.

THE RELATION BETWEEN RESPIRATION AND CATA-LASE BASED ON STUDIES OF INTACT DORMANT SEED AND SEED WITH CERTAIN MEMBRANES REMOVED. Wilmer E. Davis, Kansas State College, Manhattan, Kan. - Seeds were used with dormant embryos that permitted the removal of certain membranes that restrict the gaseous exchange. Seeds of the same lot, both intact and with membranes removed, were placed in an incubator until the respiratory intensity and catalase activity became more or less constant. The respiratory intensity was determined for a number of intact seeds and likewise of seeds with membranes removed. The catalase of each set of seeds was determined when removed from the respirometer. The ratios of the oxygen used by the intact seeds to that of seeds with membranes removed were quite similar to the ratios of catalase of the same in terms of oxygen released.

EFFECT OF THE ROOT ON CHLOROSIS IN THE GRAPE. F. B. Wann, Utah Agricultural Experiment Station, Logan, Utah.

INFLUENCE OF THE VELOCITY OF CRYSTALLIZA-TION ON THE FREEZING POINT OF LIVING TISSUES. B. J. Luyet and Sister Marguerite Sheeley, St. Louis University, St. Louis, Mo.—Determination of the osmotic pressure of living tissues is often accomplished by measuring the freezing point of the extruded sap. Freezing point determinations on the tissues themselves furnish values which are considerably lower than those of the cell sap and which are of doubtful significance. One of the factors responsible for the lowering of the freezing point in tissues is probably the low rate of crystallization. Callow has found that the velocity of crystallization decreases 350 times when one passes from water to a 3 per cent gelatin solution. If a low rate of crystallization lowers the freezing point, then gelatin solutions treated as tissues, should present abnormally low freezing points. In comparable experiments, at a moderate, constant cooling velocity, 2.5, 3.7, and 5 per cent gelatin solutions, sub-cooled to -6°C. and ice-seeded, required about 2, 12 and 45 minutes, respectively, to reach the freezing point (constant temperature level). With 7.5 per cent gelatin no freezing point could be reached and with 10 per cent the temperature did not rise at all.

DIFFERENTIAL INHIBITION OF INITIATION AND DE-VELOPMENT OF ADVENTITIOUS BUDS OF THE HYPO-COTYL OF FLAX. George K. K. Link and Virginia Eggers, University of Chicago, Chicago, Ill.—In the Bison variety of flax, decapitated hypocotyls, completely isolated hypocotyls, and hypocotyledonary segments, produce adventitious buds, some of which develop into flowering axes. Severed and set into sand the axes develop functional roots. Bud primordia of the lower hypocotyl are laid down early but do not develop further in the intact plant; those of the upper third to half, however, are not even initiated until the hypocotyl is decapitated or completely isolated. Each bud primordium, normal or

adventive, of the plant (except in cotyledonary buds) originates in a single, mature, vacuolated enidermal cell, by a transverse division without preliminary increase in cytoplasm. This division is followed by many trans- and longi-divisions. The parent cell does not enlarge until several divisions have occurred within it. It enlarges radially, mostly outwardly. One or more adjoining epidermal cells repeat this performance and become part of the primordium. Cells below the primordium divide and some of their derivatives build the vascular bridge between the vascular systems of the old and new axes. These findings, basic to our studies of the effect of auxins on cellular growth processes in bud initiation and development of flax, incidentally indicate that the adventive bud primordia of flax, derived wholly from mature, but totipotent epidermal cells. offer promising material for tissue culture experiments.

FACTORS INFLUENCING PROTOPLASMIC STREAMING IN THE OAT COLEOPTILE. R. A. Olson, Marine Biological Laboratory, Solomons Island, Md.—The intracellular translocation of substances through living cells is regulated by protoplasmic streaming. An improved method for observation of the streaming in Avena coleoptiles has been made through the use of unilateral darkfield illumination (parallel light beam, lateral light stop in condenser, darkfield stop in 4 mm. objective, water immersion between condenser and constant temperature slide, periplane ocular, coleoptile axis parallel to light beam). Precise determination of velocity of streaming can thus be obtained with ease. This method allowed the use of an objective photographic recording, depending upon the length of the streak on a film made by a moving particle during a given exposure. The effect of various substances on protoplasmic streaming has been determined. No increase in rate was caused by ethylether in concentrations below 10 g./l, by 3-indole acetic acid below 100 mg./l, by methylene blue below 10<sup>-2</sup> molar. At higher concentrations these substances decreased the velocity of streaming. KCN decreased the velocity to about 40 per cent even at concentrations of 0.01 N.

EVIDENCE RELATIVE TO THE SUPPOSED PERMEABIL-ITY OF SIEVE-TUBE PROTOPLASM. Otis F. Curtis and George N. Asai, Cornell University, Ithaca, N. Y .-In a series of articles dealing with translocation, Crafts has repeatedly stated that sieve-tube protoplasm is completely permeable and the cells are incapable of being plasmolyzed. He has proposed that this complete permeability is necessary for and favors the hypothesis that transport is brought about by a pressure flow through passive phloem. It is claimed that the lateral leakage from the permeable sieve-tubes is prevented by the surrounding living parenchyma cells. If the sieve-tubes were permeable to their contents there would probably be leakage along the phloem parenchyma walls, since these lack the casparian strip which is probably effective in preventing leakage from the permeable xylem cells of the root cylinder. Furthermore, if the phloem were

permeable to the contained sap having an osmotic concentration greater than that of the parenchyma cells, the latter would become plasmolyzed and thus open a free path for outward leakage from the phloem. We have found that the phloem exudates from Fraxinus and that from Cucurbita will strongly plasmolyze their respective phloem parenchyma cells. This, therefore, demonstrates that the sievetube membranes cannot be completely permeable to their contents. We have also found it possible to plasmolyze sieve-tubes if precautions are taken to eliminate the disruption resulting from cutting the tissues before making the tests.

INITIAL WATER-SUPPLYING POWER OF DIFFERENT KINDS OF SOIL AT ONSET OF PERMANENT WILTING IN WHEAT AND COLEUS. Burton E. Livingston and Max Wolf, Johns Hopkins University, Baltimore, Md.— At field saturation initial water-supplying power is very great for all kinds of soil, at least as great as one gram per square centimeter for the first hour. At beginning of permanent wilting in ordinary plants, its value-measured by means of porousporcelain soil-point cones with 1-hr. exposure-has been found to vary within the very narrow range between about 2 and about 7 mg./sq. cm./1st hr., being thus practically constant for all soils. This slight variability, which is not clearly related to water-holding capacity or moisture equivalent, has been regarded as probably due to experimental uncertainty of several sorts, but it is now indicated that higher values of this dynamic wilting coefficient are to be expected when the volumetric clay content of the soil is very high. In some recently completed experiments, employing wheat and coleus grown in small cylinders of somewhat loamy clay, muck humus, fine sand, and mixtures of these, the value of the coefficient was shown to be low and about the same (16 mg.-23 mg.) for humus, sand and humussand mixtures, noticeably greater and about alike (25 mg.-35 mg.) for the mixtures in which the loamy clay comprised about one-half of the soil, and much greater (72 mg.) when the loamy clay was used alone. This relation may perhaps have been due to restricted root growth in the most clavey soils.

THE DROUGHT RESISTANCE OF SOME WESTERN GRASSES. Lowell F. Bailey, Grand Rapids Junior College, Grand Rapids, Mich .- The drought resistance of Agropyron Smithii, A. ciliare, and Bromus marginatus is considered from the standpoints of (1) their ability to withstand dehydration without injury, and (2) the ability of their underground parts to remain dormant during periods of drought. Agropyron Smithii loses  $41.6 \pm 1$  per cent of its total water content before permanent wilting ensues. Bromus marginatus and Agropyron ciliare lose  $49.1 \pm 1$  and  $50.3 \pm 1.2$  per cent, respectively, of their total water contents before the onset of permanent wilting. These values indicate only a moderate ability to withstand drought without injury. The subterranean parts of all three species remained dormant during a period of six months of severe drought, and produced new shoots when water was added to the soil. After another drought period of six months' duration, only Agropyron Smithii resumed growth when water was added to the pots.

EFFECT OF RELATIVE HUMIDITY ON GERMINATION of spores of certain parasitic fungi. C. N. Clayton (Introduced by B. M. Duggar), University of Wisconsin, Madison, Wis.—Spores of Venturia inaequalis (Cke.) Wint., Ustilago nuda (Jens.) K. & S., Ustilago hordei (Pers.) K. & S., and Sclerotinia fructicola (Wint.) Rehm were discharged or dusted on clean dry cover glasses which were suspended in sealed containers above sucrose solutions, saturated salt solutions, or water in order to obtain the desired relative humidities, which were computed theoretically. Stratification of atmosphere or solution within containers was minimized and visible condensation upon cover glasses prevented, except at 100 per cent relative humidity, by mechanically rocking the containers in a bath at  $20.0 \pm 0.02$  °C. Ascospores of V. inaequalis after 48 hours in redistilled water and 100, 99.62, 99.43, 99.0, 98.65, and 98.0 per cent relative humidities averaged, respectively, 97.4, 94.4, 50.5, 23.5, 8.2, 0, and 0 per cent germination. Conidia of V. inaequalis after 24 hours in water and 100, 99.6, 99.0, 98.65, and 98.0 per cent relative humidities averaged, respectively, 96.3, 78.5, 52.2, i-14.3, 0, and 0 per cent germination. Some chlamydo-ir spores of U. hordei and U. nuda germinated at 95 per cent relative humidity, none at 93. Conidia of S. fructiola averaged 80 per cent germination in water but failed to germinate at 100 per cent relative humidity and below.

BREAKING THE DORMANCY OF BUDS OF PEAR TREES WITH GLUTATHIONE. John D. Guthrie, Boyce Thompson Institute for Plant Research, Inc., Yonkers, N. Y.—Injection of 50 mg. of glutathione in 10 cc. of water into the cut ends of branches of pear trees induced more buds to grow than on control branches treated with water. This is in agreement with similar results obtained with potato tubers (Amer. Jour. Bot. 24: 734. 1937). The stimulative action of yeast extracts on pear buds observed by Bennett and Skoog (Plant Physiol. 13: 219. 1938) may be due to glutathione.

AN EXPLANATION OF THE ADVANTAGE OF ALTER-NATING TEMPERATURES OVER CONSTANT TEMPERA-TURES IN THE GERMINATION OF CERTAIN SEEDS. Wilmer E. Davis, Kansas State College, Manhattan, Kan.—The employment of alternating temperatures in germination of seeds is especially useful in seeds with membranes that restrict the gaseous exchange. When such seeds are subjected to high constant temperatures in the germinator, both respiratory intensity and catalase activity increase. Duration of this increase depends on the temperature and the extent of the restriction of the oxygen supply; later a decline occurs until both become quite constant however long in the germinator. This condition of the embryo is here designated as fatigue and may pass into a condition of true dormancy. When alternating temperatures are employed there is a rise in both respiratory intensity and catalase activity throughout the period of germination. No perceptible fatigue is apparent. The period of time required at each temperature of the alternation depends upon the extent of the restriction of the membranes and the temperatures employed. The higher the upper temperature of the alternation may be, the shorter is the time required at that temperature, and the longer at the lower temperature in order to prevent fatigue.

A RAPID METHOD FOR DETERMINING THE GERMINA-TIVE POWER OF SOME HARD-COATED, DORMANT SEEDS. Florence Flemion, Boyce Thompson Institute for Plant Research, Inc., Yonkers, N. Y .- It has been previously shown that the viability of various dormant seeds can be determined within a short time by observing the behavior of excised embryos when placed on moist filter paper in Petri dishes at room temperature. Certain seeds, however, may have such extremely tough coats that it is difficult to remove the embryos without injury, and such seeds may also have, in addition, immature embryos. In these cases, certain pre-treatments greatly facilitate excising the embryos. Viability of such seeds, as for example, Symphoricarpos racemosus, can be determined with the following procedure: the coats are treated with concentrated sulphuric acid and the seeds are then xed in moist peat moss and kept at 5°C. for sevsal weeks until the embryos have imbibed water and developed sufficiently so that they can be excised and the viability determined within 10 days thereafter. A period of one week in moist peat moss before excision also facilitates carrying out the test for other dormant seeds such as Japanese quince, fringe-tree, etc., as well as the hard-coated chokecherry and wild plum, because not only is the excising easier but the subsequent development on moist filter paper takes place more rapidly.

The development of excised maize embryos in an atmosphere of nitrogen. Carl D. La Rue and James Merry, University of Michigan, Ann Arbor, Mich.—Observations were made on the growth of immature excised embryos of Zea mays in artificial culture on plain nutrient agar with sugar, in air and in nitrogen. The excised embryos in air showed little embryonic development but germinated and grew as small seedlings. In an atmosphere of nitrogen, the growth of the root and shoot was inhibited; the embryos did not germinate as did those in air, but showed a development more closely approximating that of the normal embryo in the ovary.

Cause of decreased Germination of bean seeds soaked in water. H. Clyde Eyster, University of South Dakota, Vermillion, S. D.—There are several experiments which prove that bacteria are not the only cause for the decrease in the germination of bean seeds which have been previously soaked in water. (1) The deleterious influence of the soaking process is much less severe at room temperature than at 10°C., at which temperature the bacteria are less active. (2) The proportion of bean seeds which germinate after having been soaked in running tap water at 13°C. for 3 days is the same as that of bean seeds which germinate after having

been soaked at 13°C. for 3 days in stagnant tap water, in which bacteria accumulate. (3) Sterilized bean seeds soaked in sterilized water germinate more poorly than control seeds. The decrease in germination of bean seeds which are previously soaked in water is due primarily to an alteration in the differential permeability of the cytoplasmic membranes. Chemical analyses of the water in which the seeds have been soaked show that there is a loss of proteins and protein constituents from the soaking seeds, and that the remaining germinative capacity of the seeds is inversely proportional to this loss.

GERMINATION OF SEED OF POA COMPRESSA L. AND POA PRATENSIS L. AT DIFFERENT ALTERNATING TEM-PERATURES. Alice M. Andersen, U. S. Department of Agriculture, Washington, D. C.—Seeds of Poa compressa and P. pratensis were sown on soil moistened with water, and on filter paper moistened with water or with N/50 potassium nitrate solution. Germination was studied at 16 different alternating temperatures for a period of 28 days. The plants were placed at low temperature for 18 hours and high temperature for 6 hours daily, except when 4 hours is stated for the high temperature. All temperature chambers were dark, except when light is stated. With seeds of P. compressa, the highest percentages of germination were obtained under the following alternating treatments: room and 30°; 15° daylight with fan and 30°; 15° daylight with fan and 30° for 4 hours; 20° and 30° daylight. In this series, about 80 per cent germination was obtained on soil, 50 per cent on filter paper moistened with water, and 90 per cent on filter paper moistened with nitrate solution. A slightly lower percentage of germination was obtained with the alternations as follows: 12° and 30° daylight; 15° daylight with fan and 35°; 20° and 30° daylight for 4 hours; 12° and 30°; 15° with fan and 30°; 10° and 30°. In these, 60 per cent germination was obtained on soil, 35 per cent on filter paper moistened with water, and 85 per cent on filter paper moistened with nitrate solution. Less favorable alternating temperatures were: 15° with fan and 30° for 4 hours; 12° and 30° daylight for 4 hours; 10° and 30° for 4 hours; 12° and 30° for 4 hours; 20° and 30°; 20° and 30° for 4 hours; 10° and 35°. In this group, about 55 per cent germination was obtained on soil, 25 per cent on filter paper moistened with water, and 80 per cent on filter paper moistened with nitrate solution. Seeds of P. pratensis gave practically the same percentage of germination with all 3 substrata. The highest percentage of germination (about 90) was obtained with the following conditions: room and 30°; 12° and 30° daylight for 4 hours; 12° and 30° dark for 4 hours; 20° and 30° daylight. A slightly lower percentage of germination (about 85 to 88) was obtained with all the other alternating temperatures, except 10° and 35°, and 15° daylight with fan and 35° which gave about 80 per cent germination.

GROWTH OF CEREAL PLANTS FROM DRY AND SOAKED IRRADIATED GRAINS. Edna L. Johnson, University of Colorado, Boulder, Colo.—Dry and soaked wheat

seeds were exposed to X-ray doses ranging from 1,000 to 60,000 r-units; barley and sorghum, to doses not exceeding 10,000 r-units. Doses up to and including 10,000 r-units did not affect survival of seedlings from dry wheat and barley seeds; 5,000 r-units given soaked grains greatly reduced survival while heavier doses were lethal; but 10,000 r-units and under, given sorghum seeds did not affect seedling survival. Noticeable height increases were apparent in all three species on exposure to 1,000 and 5,000 r-units. Three groups of dry wheat seeds, one each of soaked wheat, dry and soaked barley, and soaked glume-free sorghum seeds exceeded controls both in height to tip of highest leaf and to tip of head. Plants from dry, glume-free sorghum seeds given 1,000 r-units were taller than the controls. Tillering was considerably increased in practically every group of wheat plants which survived the given dose. Barley from soaked seeds tillered slightly more than the controls. Sorghum plants from glumefree irradiated seeds had a high percentage of plants with more than one main shoot.

FLOWER PRODUCTION IN DIGITALIS AND CRASSULA RUBICUNDA BY LOW TEMPERATURE AND LIGHT. John M. Arthur, Boyce Thompson Institute for Plant Research, Inc., Yonkers, N. Y .- Both Digitalis and Crassula rubicunda were grown in the greenhouse with night temperatures as low as 55°F. for several years without flowering. At this temperature, flowers were not produced under either long days or short days. Digitalis plants set out-of-doors in September came into flower the following June. Plants transferred to an unheated greenhouse on January 11 flowered in June. When the plants were held in a cold room at 50°F. each night and kept in the greenhouse each day from May 14 until September 30, and then given a long day and a night temperature of 55° to 60°F., flowering occurred in November. Flowers were produced on Crassula rubicunda by similar cold-night treatments except that the low temperature was given from November 14 until April 26. One plant removed from the cold treatments in February produced vegetative branches but no flower buds.

THE ABSORPTION SPECTRA OF WATER EXTRACTS OF SEVERAL SPECIES OF PHOTOSYNTHETIC PURPLE BAC-TERIA. C. S. French, University of Chicago, Chicago, Ill. (Introduced by J. T. Buchholz, University of Illinois, Urbana, Ill.).—In purple bacteria the pigments which absorb the light used for photosynthesis exist in solution combined with a protein. Treating a suspension of these bacteria with supersonic vibration breaks the cell wall to liberate the juice containing two pigments, bacteriochlorophyll and a carotinoid. Both are attached to a protein which makes these substances soluble in water when they are otherwise soluble only in organic solvents. This complex, called photosynthin, can be precipitated and redissolved by changing the salt concentration or the pH of the solution. Such solutions will act as photocatalysts for oxidation of ascorbic acid. Extracts have been prepared from four species, Spirillum rubrum, Rhodovibrio sp., Phaeomonas sp., and Streptococcus varians, and their absorption spectra measured in the visible and near infra-red. The main band of the bacteriochlorophyll occurs at 875, 855, or 840 m $\mu$  depending on the species, while its secondary bands are at 790 and 590 m $\mu$  in all the bacteria examined. The position and height of the bands are the same in the live cells and in the extract. The ability of the organisms to carry on photosynthesis is believed to depend on the properties of this chromoprotein.

Use of the dropping mercury electrode in PHOTOSYNTHESIS STUDIES WITH CHLORELLA PYRE-NOIDOSA. W. E. Moore and B. M. Duggar, University of Wisconsin, Madison, Wis.—The following are among the many advantages of the method discussed: no pre-treatment, smaller amount, and less concentration of algae required; accurate measurement of transmitted light; very rapid; apparatus simple in manipulation, flexible, and portable; small changes measured; special nutrients may be used; duplicate set-ups for simultaneous runs; and at any point the oxygen level is instantly known. Suggestions to aid in the successful application of the method are included in this report. With a number of culture solutions and under different acceptable physical conditions for growth there is little variation in the values for the quantum efficiency. The efficiency as measured is lower than the value reported by Warburg. Data for the quantum efficiency under the following conditions are presented: rate of growth; various inorganic media (Warburg, modified Warburg, Emerson, sea-water, A-Z in water, soil extract, city water, tin distilled water, Pyrex distilled water, and lake water); organic additions to the culture; different light intensities for growth and for the efficiency measurement; effect of the transfer from high light to low light intensity; efficiency from two light intensities alone with no dark period; algae from different sources; attempts to kill the cells and continue the run.

THE CONTRIBUTION TO PHOTOSYNTHESIS OF LIGHT ENERGY ABSORBED BY CAROTENOIDS IN THE DIATOM NITZSCHIA CLOSTERIUM. Herbert J. Dutton and Winston M. Manning (Introduced by B. M. Duggar), University of Wisconsin, Madison, Wis.—A study has been made of the quantum efficiency of photosynthesis in the marine diatom Nitzschia closterium, using low intensity light of wave lengths 660, 546. 436 and 405 m $\mu$ . In conjunction with these experiments, a quantitative study was made of the light absorption by the different pigments in this diatom. In contrast to green algae, N. closterium was found to contain a large amount of fucoxanthin but little or no chlorophyll b. The quantum efficiencies were found to be of the same order of magnitude as those found in this laboratory for the green alga Chlorella. A greater quantum efficiency for photosynthesis in N. closterium was found at wave lengths where a large fraction (30 to 70 per cent) of the absorption was due to fucoxanthin and other carotenoid pigments than at wave lengths where nearly all of the

light was absorbed by chlorophyll. These results strongly indicate that light absorbed by pigments other than chlorophyll can be utilized by this diatom

in photosynthesis. EFFECTS OF MONOCHROMATIC ULTRAVIOLET RADIA-TION ON THE GROWTH OF FUNGOUS SPORES SURVIVING IRRADIATION. Albert E. Dimond and B. M. Duggar, University of Wisconsin, Madison, Wis.—Spores of Rhizopus suinus Niels, were exposed to varying dosages of radiation of wave length 2,650 Å., and measurements of the growth of survivors were made under three sets of conditions. First, photomicrographs were made of hyphae in hanging drops at frequent time intervals, and, after enlargement, the areas were determined with a planimeter. Growth curves of individuals over 4-10 hours were established in this manner. The growth response of irradiation survivors was much more variable than was that of unirradiated individuals; also a number of hyphae from irradiated spores grew at a significantly more rapid rate. Second, the growth on Petri dishes of colonies from irradiated spores was compared with that of unirradiated spores. During the time period of 1.5 to 30 hours after the beginning of incubation, the growth rate of the two types of mycelium was identical. Third, comparatively dry weights of mycelial mats produced in liquid culture were followed. From 36 hours onward, growth of the two types of culture proceeded at the same rate. However, growth continued for a longer period in cultures produced

PHOTOCHEMISTRY AND ABSORPTION SPECTROSCOPY OF THE PYRIMIDINE COMPONENT OF VITAMIN B<sub>1</sub>. Fred M. Uber and Louis A. Fourt, University of Missouri, Columbia, Mo.—Ultra-violet absorption curves for 2-methyl-5-ethoxymethyl-6-amino-pyrimidine show a marked dependence on pH, exhibiting two distinct maxima for a pH of 7.0 or more but only one maximum for a pH of 6.4 or less. The positions of the maxima shift with pH. Molecular extinction coefficients vary greatly with molar concentration, particularly for solutions less than 0.000,1 molar. Beer's law is not obeyed at a pH of either 4.8 or 7.8, and presumably not at others. Irradiation of phosphate buffered solutions, pH 4.8 and absorption maximum of 2,480 Å, resulted in a rapid decrease of the selective absorption. This photochemical decomposition of the pyrimidine has been found to yield ammonia nitrogen.

by irradiated spores. This resulted in the production

of fungous mats which were 20 per cent heavier than

mats from untreated spores.

ULTRA-VIOLET INACTIVATION OF THE PYRIMIDINE COMPONENT OF VITAMIN B<sub>1</sub>. Frank Verbrugge and Fred M. Uber, University of Missouri, Columbia, Mo.—Inactivation of 2-methyl-5-ethoxymethyl-6-amino-pyrimidine has been brought about by irradiation at a wave length of 2,537 Å. The irradiated solution had a concentration of 0.000,125 molar and was maintained at pH 4.8 in a phosphate buffer. Tests for inactivation were made with cultures of Phycomyces Blakesleeanus, 0.05 cc. of the above solution being added to 250 cc. of nutrient medium

and then aliquotted to 10 flasks, which were inoculated with sterile spores of the organism. Thiazole was present in all flasks at a constant concentration, which was twice the molar concentration of the non-irradiated pyrimidine used as a control. Growth curves of dry weight of cultures as a function of pyrimidine concentration gave a basis for the estimation of the inactivation. These curves were linear in the range  $0.0125-0.15 \times 10^{-7}$  molar for cultures grown for 10 days at  $24^{\circ}\mathrm{C}$ .

EFFECT OF LIGHT OF DIFFERENT WAVE LENGTHS ON PLANT GROWTH. F. W. Went, California Institute of Technology, Pasadena, Calif.—Leaf growth, growth in length, phototropism and chlorophyll formation in etiolated pea seedlings is greatly affected by small amounts of light. The spectral sensitivity of each of these processes is very different, indicating that different pigments and different lightsensitive reactions are involved in each process. Thus leaf growth is most pronounced in red, less in yellow and blue, and still less in green, so that its absorption spectrum resembles that of chlorophyll. Growth in length is most decreased by yellow, and least by blue light. Phototropism only occurs in blue and green, confirming older work, and indicating light absorption by carotenoids. Chlorophyll formation is equal in red, vellow and green, but distinctly less in blue.

EFFECTS OF LIGHT QUALITY ON SERRATIA MARCES-CENS. K. W. Kreitlow (Introduced by Lewis H. Flint), Louisiana State University, University, La. -Radiation studies on Serratia marcescens were carried out by growing tube cultures of the organism on Difco nutrient agar and exposing the cultures to General Electric red, blue, and daylight fluorescent lamps. Controls were kept in darkness by wrapping the culture tubes in black photographic paper. Seven isolates of Serratia marcescens were used in these studies and all were found to respond similarly under any particular light quality. The most intense pigmentation was found in cultures grown in the dark and under red light. Less pigmentation occurred in tubes grown under daylight or white light. Cultures grown under blue light gave very slight to no pigmentation whatsoever. Best results were obtained after three or four serial transfers of the organism under light of a particular quality. Maximum gain or loss of pigment resulted after 72 hours of exposure to light of any quality with the cultures being incubated at 20°C. Decrease in pigmentation was also observed when cultures were grown in plain glass tubes exposed to ultra-violet radiation; this loss of pigment was probably due to short blue rays since exposure under filters transmitting only ultraviolet radiation gave almost normal pigmentation.

AUXIN CONTENT IN OVARIES AND YOUNG FRUITS AT DIFFERENT STAGES OF DEVELOPMENT. F. G. Gustafson, University of Michigan, Ann Arbor, Mich.—Various stages of young ovaries and fruits have been used for this study. It may be said that in general the ovaries in the flower bud stage contain more auxin per unit weight of material than do the ovaries

of the flower, and as the ovary develops into a fruit there is usually a further decrease.

AUXIN CONTENT IN CUSCUTA AND ITS HOST. F. G. Gustafson, University of Michigan, Ann Arbor, Mich.—Extraction of the growth hormone with ether showed that the part of the host actually attacked by the Cuscuta had a much higher growth hormone content than that part not attacked by the parasite. That part of the Cuscuta actually in contact with the host had also a higher auxin content than the young free part of the parasite.

Some chemical agents in the production of OVERGROWTHS ON PLANTS. Michael Levine, Montefiore Hospital, New York, N. Y .- The effects of indole butyric acid and a-naphthalene acetic acid were studied on a large series of plants such as Ricinus, Cosmos, Helianthus, Lycopersicon, Datura, Brassica oleracea botrytis, Nicotiana glauca, Rosa, Tagetes erecta, and Kalanchoe Daigremontiana. Tryptophane, indole, and a mixture of tryptophane and Phytomonas tumefaciens were also tested on a similar group of plants. Indole butyric acid and a-naphthalene acetic acid produce massive reactions without roots on decapitated sunflower and Jimson weed. On Kalanchoe, broccoli, and Cosmos, these chemicals induce intumescences with roots. Scalelike organoids appear on the Kalanchoe and broccoli. Tryptophane and indole produce small globular masses on the broccoli and N. glauca, while a mixture of tryptophane and P. tumefaciens produce discrete crown galls. Suspensions of Sudan III, Sudan IV, p-aminoazo-benzene, 2-amino-5-azotoluene, and Grubler's scharlach red were also tested on similar plants. These dyes induce only swellings; yet some of these chemicals are known to be carcinogenic for animals. Heteroauxin-induced overgrowths, under microscopical examination, show close resemblance to crown gall tissue. These "chemical tumors" represent a protective mechanism against an irritating agent.

THE RELATION BETWEEN RESPIRATION, PROTO-PLASMIC STREAMING, AND AUXIN TRANSPORT IN THE AVENA COLEOPTILE. H. G. du Buy and R. A. Olson, University of Maryland, College Park, Md.—The hormone phenomena of vernalization are dependent upon translocation of certain substances through living cells. Protoplasmic streaming regulates the intra-cellular part of translocation; respiration regulates the streaming velocity, and thus the translocation. The Heyrovsky polarographic method has been adapted to a dual microrespirometric method for oat coleoptiles. A marked change in respiration rate below a certain O2-tension has been observed, the critical oxygen tension varying with age. Similar age-effects have been found by others for protoplasmic streaming. Since the reduced rate of respiration after KCN inhibition parallels that of the reduced respiration rate below the critical tension, it is suggested that the respiration system operating at low O2-tension and that not inhibited by KCN are the same. 2-4-dinitrophenol inhibits both the rate of respiration and streaming to the same degree. 3-indole acetic acid alone or in the presence of fructose has no effect on respiration except above 100 mg./l. where it decreases respiration and streaming. Since the protoplasmic streaming persists at the lower respiration rate below the critical oxygen tension, the streaming does not depend upon one specific respiratory system. Direct transport measurements in oat coleoptile cylinders treated with KCN show that the transport of growth regulators is decreased to the same extent as the respiration and the streaming. The concept concerning translocation put forward in Biodynamica 36 (1938) obtains herewith further support.

EFFECT OF VARIOUS GROWTH REGULATING SUB-STANCES ON THE GROWTH OF LETTUCE SEEDLINGS (VARIETY GRAND RAPIDS). R. B. Stephenson (Introduced by H. G. du Buy), University of Maryland, College Park, Md.—Lettuce seedlings are cultured (1) as whole plants, (2) roots and shoots separately, (3) roots and shoots in the same flasks, in sterile media to which special substances have been added in concentrations of 0.1 and 1 mg./l. Thiamin and nicotinic acid-treated roots have nearly the same total growth, but branching in nicotinic acid (and naphthylacetamid) is profuse, and minimal in thiamin. Thiourea on roots alone has little effect, but on entire seedlings it produces the largest root system of all series. Naphthylacetamid has the same effect, but less pronounced. Thiourea definitely retards the growth of a pathogenic Fusarium sp. added to the cultures. Roots treated with naphthyl and indole acetic acids are markedly inhibited in their elongation but produce the greatest number of lateral root primordia. Treated shoots produce from two to three times the normal number of adventitious root primordia. When roots and shoots are cultured together, fewer root primordia are formed on the shoots than on shoots which are isolated from the roots; whereas the number induced on the roots attached to shoots is greater than on the isolated roots. Elongation of the roots is inhibited by these substances, but swelling of the roots occurs to produce what appear to be artificial root nodules. This effect on root culture makes a simple test to distinguish "true" auxins.

THE PRODUCTION AND INHIBITION OF ADVENTI-TIOUS ORGANS AND SOME EFFECTS OF GROWTH SUB-STANCE VAPORS. P. W. Zimmerman and A. E. Hitchcock, Boyce Thompson Institute, Inc., Yonkers, N. Y.—In experiments designed to study initiation and inhibition of adventitious organs, disbudded stem cuttings of Hibiscus syriacus were treated with vapors of growth substances and placed in moist chambers. Both normal and disbudded, non-treated, cuttings served as controls. Controls with normal buds did not form adventitious shoots while disbudded cuttings initiated numerous adventitious shoots, associated with the region of lenticels or callus. As many as 75 adventitious buds were initiated on one six-inch cutting, the majority arising from the distal region. A few adventitious roots arose from the basal end, some protruding through the lenticels. The upper shoots grew fastest and finally inhibited growth

of other adventitious shoots below. The influence appeared to be of a regulatory nature and due to a natural chemical substance. Cuttings treated with vapors from esters of phenylacetic, indolebutyric, indoleacetic,  $\alpha$ -naphthaleneacetic,  $\beta$ -naphthoxyacetic acids,  $\alpha$ -naphthaleneacetamide, or  $\beta$ -naphthoxyacetamide showed increased callus formation at wounded areas, proliferation of lenticels, retardation or inhibition of adventitious shoots, and induction of adventitious roots associated with proliferating lenticels and callus. The vapors of indole and naphthalene compounds induced roots from upper middle and lower regions of the cuttings, thus disturbing normal polarity and in contrast with adventitious shoot initiation in controls.

COMPARATIVE ROOT-INDUCING ACTIVITY OF IN-DIVIDUAL SUBSTANCES AND MIXTURES. A. E. Hitchcock and P. W. Zimmerman, Boyce Thompson Institute, Inc., Yonkers, N. Y.—Combinations of IB (indolebutyric), IA (indoleacetic), and NA (naphthaleneacetic) acids in talc were applied in proportions of 0:4, 1:3, 2:2, 3:1, 4:0, 1:1:1, and 2:1:1 in total concentrations of 2, 5, and 12 mg./g. to cuttings of chrysanthemum, Euonymus, Hibiscus, and privet. IB, IA, NA, and PA (phenylacetic acid) were applied individually (4 to 64 mg./l.) and with additions of 8 or 16 mg./l. of another substance. Increasing the proportion or concentration of a substance most active for a given species generally increased the average number of roots per cutting. IB-NA mixtures were more effective for all species than IB-IA or IA-NA mixtures. Mixtures were more active or active over a broader range than individual substances. The activity of IB or NA solutions (4 to 28 mg./l.) was increased by the addition of 8 or 16 mg/l. of a substance which individually showed little or no activity (PA) up to 64 mg./l. These results are not explainable on the basis of a proportional additive effect, dilution, or inactivation. The effectiveness of mixtures over a broader concentration range without injury and for more species as compared with single substance preparations indicates a possible practical use of such mixtures for rooting cuttings.

FURTHER STUDIES ON THE VEGETATIVE PROPAGA-TION OF SOME GYMNOSPERMS, WITH INDOLE ACETIC ACID. Albert L. Delisle, College of William and Mary, Williamsburg, Va.—Experiments were made on rooting cuttings of some forest and cultivated Gymnosperms, which included representative members of all the tribes of the Coniferales. Particular emphasis was placed on Abies concolor, Abies pectinata, Abies koreana, Picea pungens, Picea Omorika, Sequoia sempervirens, Sciadopitys verticillata, Tsuga diversifolia, Taxus spp. and var., Podocarpus neriifolia and Ginkgo biloba. Of the above, with one or two possible exceptions (Ginkgo and Sciadopitys) it was found that auxin greatly stimulated rooting. The rooting medium and especially the time of year at which cuttings were made determined in large measure the percentages of rooting. For most, best results were obtained with cuttings taken in the

late Fall and Winter, and the poorest when taken in mid-Summer. Except in cuttings of *Picea pungens*, a sand and peat mixture was optimal for rooting. A practical method is offered for the successful vegetative reproduction of the above named members of the Coniferales.

THE HORMONE CONTENT OF MAIZE ENDOSPERMS AS DETERMINED BY DIFFERENT EXTRACTION METHods. G. S. Avery, Jr., H. B. Creighton, and B. Shalucha, Connecticut College, New London, Conn.-Endosperms of Canada Flint maize were removed from dormant and germinating grains at following time intervals: air dry dormant seeds, ½ day, 1 day, 3 days, 5 days, and 7 days. Two different extraction methods were used: (1) A known weight of tissue was ground with sand and extracted with (a) chloroform (Thimann, 1934), (b) alcohol (Laibach and Meyer, 1935; Avery, 1939), and (c) water (Chlodony, 1935; Overbeek, 1938); (2) a known weight of tissue was placed directly in ether and allowed to stand overnight at ca. 5°C. (Boysen Jensen, 1937; Overbeek, 1938). In all instances the extract was taken up in water, then mixed with agar and the hormone content determined by the deseeded Avena test (Skoog, 1937). Water extracts give the maximum yield of growth hormone from dormant endosperms and those at all stages of germination. Significantly different amounts are obtained by the various alcohol methods and the chloroform method. The greatest differences in yield by the various extraction procedures are found in the first 24 hours of germination. Subsequent differences in yield are not as great, but regardless of method, the yield decreases as germination progresses. At the end of seven days very little growth hormone can be detected by any of the methods used.

Growth hormones and heterosis. G. S. Avery, Jr., H. B. Creighton, and B. Shalucha, Connecticut College, New London, Conn.—One possible approach to a physiological explanation of hybrid vigor is through a study of growth hormone content of (1) dormant seeds, (2) seedlings, and (3) older plants which exhibit heterosis. Preliminary studies on dormant endosperms of selected inbred lines of maize show no characteristic relationship in hormone content between parents and hybrid, on a per endosperm basis. On the basis of hormone content per gram of endosperm, the F<sub>1</sub>s are intermediate between the parents.

AUXINS OF HYALOPTERUS ARUNDINIS AND OF ITS HOST. George K. K. Link and Virginia Eggers, University of Chicago, Chicago, Ill.—Comparative quantitative and qualitative studies were made of the auxins of the aphid Hyalopterus arundinis and of the leaf of Phragmites communis on which it feeds. On the basis of wet weight, the ether extract of the aphid in Avena coleoptile tests is 7–25 times, and on the basis of dry weight, 10–37 times more potent than the leaf extract. Per gram of water, the aphid extract is 3–18 times more potent than that of the leaf. Water, acid, and alkali refluxing, together with color tests of the extracts, indicate that the leaf and

aphid extracts each contains the same kind of auxins but in different proportions. Auxins which behave like indole-acetic acid are very much more abundant in the aphid extract. This difference may have its basis in auxins of the honeydew. In view of the fact that sections of the leaf indicate that the aphid derives most of its food from the phloem, the results may indicate that the phloem is the main site of auxins in the leaf of *Phragmites communis*.

DISTRIBUTION OF AUXIN IN SUBTROPICAL FRUIT PLANTS. William C. Cooper and Kenneth R. Knowlton, U. S. Subtropical Fruit Research Station, Orlando, Fla.—Using Van Overbeek's ether extraction method and Went's Avena test, auxin determinations were made on different parts of Citrus and pineapple plants In Citrus the main center of auxin production appeared to be in the growing bud with its embryonic leaves, old leaves producing little or none. The developing fruit also produces auxin. The auxin in the stem below a fruit or below a flush of new growth was shown to be supplied by the fruit or the flush growth. In the pineapple the center of auxin production appears to be in the embryonic etiolated leaves in the heart of the plant. The ethylene treatment to induce blooming in the pineapple had no marked influence on the distribution of auxin in the plant.

PRODUCTION OF AUXIN BY TOBACCO CALLUS CULTURED IN VITRO. Folke Skoog, Harvard University, Cambridge, Mass.—A tobacco callus culture obtained from Dr. Philip White has been propagated on his synthetic liquid and solid media over a period of seven months in the light, and others for five months in the dark. Determinations of auxin made at successive stages of growth of the cultures show that the callus synthesizes auxin both in light and in darkness. The obtainable amounts of active substance vary with cultural conditions and with the age of the cultures.

Some effects of wounds and wound hormones. Carl D. LaRue, University of Michigan, Ann Arbor, Mich.—Bacterium tumefaciens produces large tumors on leaves of Kalanchoe rotundifolia. If inoculated leaves are wounded repeatedly by needle pricks the tumors grow much more rapidly than on unwounded leaves. Hormone extracts prepared according to Bonner's method and applied in lanolin or by injection, gave similar results. Tumor tissue was induced by wound hormone extracts, but definite tumors did not form. Periderm formation was induced in unwounded stems of Coleus by the application in lanolin of extracts containing wound hormones. Tyloses are readily induced in young twigs of Morus alba by wounding. They have been produced also by placing the bases of long shoots in a solution containing wound hormones. Control plants developed tyloses only near the basal cut, but those in the wound hormone solution produced tyloses far up the shoots. Living twigs of Hibiscus placed in nutrient solutions with sugar stimulate growth of cells from intumescences of the Hibiscus rosa-sinensis. In the absence of living twigs, growth is secured by the addition of extracts containing wound hormones. Wounding induces root formation in petioles of Coleus, stems of willow, Mentha arvensis, and Sedum sp. Extracts containing wound hormones give similar results.

THE INFLUENCE OF THE STAGE OF THE REST PERIOD ON ROOTING AT THE CUT SURFACE OF POTATO TUBERS. John D. Guthrie, Boyce Thompson Institute for Plant Research, Inc., Yonkers, N. Y.—Pieces of old or non-dormant potato tubers form roots at the cut surface more readily than pieces of freshly harvested or dormant potato tubers when treated with neutralized indole-acetic acid. Freshly harvested tubers rendered non-dormant by treatment with ethylene chlorohydrin form roots more readily than untreated controls.

FURTHER STUDIES ON GROWTH SUBSTANCES IN RE-LATION TO THE MECHANISM OF THE ACTION OF RADIA-TION ON PLANTS. H. R. C. McIlvaine and H. W. Popp, The Pennsylvania State College, State College, Pa.—Previous studies (Jour. Agr. Res. 57: 931-936. 1937) were continued with particular reference to the action of regions of the visible spectrum. Brassica Rapa L. seedlings were grown for 31/2 or 7 days, and periodically irradiated under varying conditions. The sources of radiation were (a) daylight, (b) a 500-watt Mazda lamp, (c) a G. E. mercury vapor lamp in glass, and (d) a mercury vapor lamp in quartz. Corning glass filters were used, and, where possible, exposures were such as to equalize the total energies reaching the plants. Exposures to varying conditions of daylight and darkness were also made. At the end of the series of treatments the amounts of growth substance in the seedling tips were determined by a modified Went Avena curvature technique. Records of the mean heights of the seedlings indicated a positive correlation between seedling height and the amount of growth substance evidenced in the test. In all cases, results showed that when seedlings are subjected to the shorter visible wave lengths of radiation, the height is less, and there is a smaller amount of growth substance present. Two series of experiments indicated that the growth substance is inactivated to an appreciable extent by daylight, and that it tends to accumulate in darkness.

Note regarding trimethylamine as a plant sex hormone. Lewis H. Flint and Fred McGoldrick, Louisiana State University, University, La.—The injection of potato tubers with aqueous solutions of trimethylamine, carried out as a student's problem in a course in advanced plant physiology, gave results of unusual interest relating to the differentiation of flower buds, the production of blooms and the development of secondary flowering from adjacent axillary shoots. The results obtained tended to confirm those reported by Professor Havas of Belgium in the Science News Letter of November 19, 1938, concerning the efficacy of trimethylamine as a sex hormone.

MAINTAINING A HIGH POTENTIAL GROWTH RATE IN AVENA COLEOPTILES BY PRE-TREATMENT WITH AUXIN. Charles L. Schneider, Harvard University, Cambridge, Mass .- If Avena coleoptiles are pre-treated with low concentration of auxin, then on subsequent treatment with high concentration of auxin the maximal growth rate is as much as fifty per cent higher than that of controls. By using the maximum angle curvature of the Avena test, the increase is enhanced to as much as two hundred per cent. Especially for the maximum angle assay method, there is a sharp optimum curve for the concentration used in pretreatment, the peak being at about 0.02 milligrams of indole-3-acetic acid per liter of agar, that is, at the concentration giving about ten degrees curvature in the Avena test. This kind of pre-treatment is to be distinguished from the "hemi-auxin" pre-treatment of Went, for it achieves the opposite resultsit increases the maximum angle but not the sensitivity to low concentrations. The effectiveness for pretreatment of chemically different auxins is a function of their activities; for the less active auxins, not only is the minimal effective concentration higher, but also the optimal response lower.

HISTOLOGICAL AND ANATOMICAL CHANGES INDUCED BY INDOLE-ACETIC ACID IN ROOTING CUTTINGS OF PINUS STROBUS. Albert L. Delisle, College of William and Mary, Williamsburg, Va.—Cuttings and

brachyblasts from 4-year-old seedlings root readily with auxin treatment. The hormone causes great increase in the size of the cuttings by stimulating cell division and cell enlargement in certain tissues. The cells of the cortex, and those still remaining of the pericycle, initiate mitotic divisions, soon obliterating the cortical canals by the pressure of the adjacent dividing cells, thus contributing largely to the increase in the diameter of the cutting. A periderm is produced subepidermally, and this gives rise to the callus. The cambium is stimulated to form much secondary tissue, consisting of xylem, phloem and parenchyma. Cambial activity, however, is not uniform throughout. Opposite a congerie of rays, adjacent to the leaf trace, cushions of meristematic cells appear which continue dividing, and soon produce the root primordia which by further cell divisions push through the phloem, cortex and periderm, to the outside. The pith is unchanged. Differences in rooting ability between corresponding cuttings of young and old trees seem largely of a physiological nature. Similarly, auxin treated brachyblasts of young and old trees show marked differences in rooting. Unlike rooted cuttings, rooted brachyblasts fail to survive unless adventitious buds have previously been caused to develop in them.

### SYSTEMATIC SECTION

Abstracts of the Papers Presented before the Systematic Section of the Botanical Society of America, Columbus, Ohio, December 28 to 30, 1939

Notes on the Michigan algae. Alton H. Gustafson, Williams College, Williamstown, Mass.—A number of additions to the algal flora of Michigan have been made. Summary records of the algal population including data from several previously uncited papers are assembled. Comparisons are made with the flora of other regions, especially that of Massachusetts.

NEW MOTILE ALGAE FROM NEW JERSEY. Harold C. Bold and Tracy E. Hazen, Columbia University, New York, N. Y.-In collections from a small pond in New Jersey the authors found two motile organisms which appear to be new. They are both of primitive organization since they lack a firm cell wall; they are, however of very different affinities. The first clearly belongs to the class Euglenophyceae and is probably related to the genus Eutreptiella. It differs from Euglena in the possession of two flagella and in the absence of a "gullet" and stigma. Under certain conditions its metaboly is so pronounced that the movement can be considered almost amoeboid. The second organism is a member of the class Chlorophyceae, family Polyblepharidaceae and is probably a new species of the genus Tetrachloris Pascher and Jahoda, which genus has not been reported before from the United States. The present species is significant in that it brings together characters found in diverse genera and is therefore probably more generalized. This genus Tetrachloris may well be regarded as ancestral to the well known Carteria.

A NEW RECORD OF DISTRIBUTION FOR THE GENUS OF OCCLADIUM. Glenn C. Couch, Ohio State University, Columbus, Ohio.—The range of distribution in this country for the algal genus Oedocladium has appeared to be limited to the Atlantic Coastal States. The discovery of the genus in the Boston Mountains of Arkansas greatly extends its range.

AN OSMUNDA HYBRID. R. M. Tryon, Jr., Gray Herbarium, Cambridge, Mass.—Three sheets of Osmunda in the Gray Herbarium have been identified as O. Claytoniana L. × regalis L. var. spectabilis (Willd.) Gray. This is the first instance the author has been able to find of a hybrid in the genus Osmunda. The hybrid has characters of both parents, and some intermediate between them. Also, many of the sporangia are abortive.

THE PALMACEAE: A UNIQUE TAXONOMIC PROBLEM. Miriam L. Bomhard, U. S. Forest Service, Washington, D. C.—An outline chart of pertinent data and the minimum herbarium requirements necessary for the accurate identification of palms.

A NEW SPECIES OF HARTMANNIA. W. H. Horr, University of Kansas, Lawrence, Kan.—Closely resembling H. speciosa (Nutt.) Small, the plant has been collected in eastern Kansas and western Mis-

souri, the main difference being that it is smaller than *H. speciosa* and that the flowers open pink and fade pink. Ten years of reproduction from seeds have brought no noticeable changes.

DESCRIPTIVE METHOD OF LINNAEUS' SPECIES PLANTARUM. Henry K. Svenson, Brooklyn Botanic Garden, Brooklyn, N. Y.—A brief analysis of the components of the Linnaean "descriptions" and relationship to pre-Linnaean works.

A NEW BEGINNING DATE FOR BOTANICAL NOMENCLATURE. Louis Cutter Wheeler, University of Missouri, Columbia, Mo.—Such a burden of taxonomic and nomenclatural synonymy has accumulated since Linnaeus' Species Plantarum that it is time to consider a new starting date for Botanical Nomenclature. To eliminate the present accumulation of useless synonymy and impose a simplified nomenclature on the science of botanical taxonomy within a single generation would require a second Linnaeus.

A series of uniform monographs, much like Das Pflanzenreich, but executed with strict regard for the Rules of Botanical Nomenclature and the type concept, would be bibliographically the most convenient, and most likely to be universally accepted after the approval of the work by an international body of systematists. Each monograph would establish a new starting date for the nomenclature of the group treated. The change of beginning dates must be voluntarily accepted rather than thrust upon us by any man or small group of men. International participation in the basic monographs would encourage general cooperation. There are many problems to solve before any plan for a new beginning date can be put into operation, but now is the time to begin. Perhaps by the end of the second century of binary nomenclature we shall have commenced clearing our nomenclatural attic.

A STATISTICAL APPROACH TO YUCCA WHIPPLEI. Carl Epling and A. L. Haines, University of California, Los Angeles, Calif.—A method of field study, based upon that employed by Anderson on Iris, is described. The geographic variation of Yucca Whipplei is analyzed and five geographic races or subspecies are described.

Variations of ophioglossum vulgatum. Robert T. Clausen, Cornell University, Ithaca, N. Y.—As the result of the study of 555 lots of specimens of Ophioglossum vulgatum L., there seems no sound basis for the segregation of subspecies correlated with geographical distribution. The characters available for such segregation appear not fundamental. Shape and size of sporangia vary with the age of the fertile segment. Presence or absence of a basal sheath depends on the degree of weathering (rotting) of the leaf stalk of the previous season and, in the case of herbarium specimens, on the degree of

washing to which the plants have been subjected. Translucence of the blade depends on age. Shape of the blade appears as the best character available, but this is scarcely sufficient for subspecific segregation. Professor Fernald, in a recent publication, the positiveness of which would seem unlikely had it been based on vast series of plants from throughout the range of the species, recognized four varieties, but these several variations occur too generally, both in the Old and New Worlds, to make practical their recognition as geographical races.

VIBURNUM PUBESCENS, A POLYMORPHIC SERIES. H. A. Gleason, New York Botanical Garden, New York, N. Y.—V. pubescens is primarily a coastal plain derivative of the Appalachian V. dentatum, varying greatly in the size, shape and texture of its leaves and the distribution and amount of pubescence. It also occurs inland, where it has presumably

hybridized with Viburnum molle.

ORIGINS AND RELATIONSHIPS OF THE FLORA OF A BARRIER RANGE OF CALIFORNIA. Joseph Ewan, University of Colorado, Boulder, Colo.-The San Gabriel Mountains, rising chiefly in Los Angeles County, are a fault-block barrier range, sixty miles long by five to twelve miles wide, lying between the coastal plain and the Mohave Desert. Its flora represents seven components, viz., panarctic, Sierran, Monican, Mohavan, Mexican, Rocky Mountain, and endemic elements. The panarctic element is the weakest today, whereas the Sierran is the strongest. The endemic group includes twelve species and nine subspecies; these have affinities with the Bernardine region to the south and the Coast Ranges to the north, but most often represent plant aborigines which have developed in situ, this range being open for population before adjacent areas. The geomorphogeny of the range has played a decisive part in the history of the flora, strongly delimiting the species groups within the range so that eight floristic subareas may be recognized, each with a distinctive geologic history. There are manifest floristic relationships with the Santa Monica, Tehachapi and San Bernardino ranges and also with the distant Santa Lucia and Napa ranges, and the Rocky Mountains via Great Basin connections.

Some problems of plant distribution in Northwestern washington. W. C. Muenscher, Cornell University, Ithaca, N. Y.—Results of field studies made during the summers of 1937 and 1939, indicate that the distributions of many plants west of the Cascade Mountains are limited more by the action of climatic and physiological factors than by physical barriers against migration.

Phytogeographical notes on the arbuckle mountains of oklahoma. Milton Hopkins, University of Oklahoma, Norman, Okla.—In the last two years the identity of numerous plants formerly thought not to occur north of Texas has been ascertained. Their discovery in this calcareous area lends additional evidence to the theory that the flora here is an ancient one closely related to that of the Edwards Plateau of southwestern Texas which is also

of calcareous composition. Previously, few plants were known which were common to both regions of limestone but recent studies have added, among numerous others, Cercis reniformis, Quercus texensis, Fraxinus texensis, Rhus Copallina var. lanceolata, Psoralea Reverchonii and Abutilon incanum.

THE TAXONOMY AND DISTRIBUTION OF THE GENUS LYCOPERSICON. Cornelius H. Muller, Bureau of Plant Industry, Washington, D. C.—The tomatoes, comprising the genus Lycopersicon, are very closely related to the genus Solanum, but the two genera are readily distinguished from one another. The species of Lycopersicon fall naturally into two subgenera, the edible red-fruited species and the unpalatable green-fruited species. The green-fruited group is confined to western South America while the red-fruited group has been spread widely as a result of cultivation. Evidence points to the conclusion that Lycopersicon esculentum var. cerasiforme, the cherry tomato, is the wild prototype of the common Lycopersicon esculentum of horticulture.

Taxonomy of symphoricarpos. George Neville Jones, University of Illinois, Urbana, Ill.—A genus of the Caprifoliaceae with about 15 species, one Chinese, the others North American and distributed from Alaska to Guatemala. The morphological characters of taxonomic and phyletic value are color and size of fruits, shape and size of corollas, leaf-shape, pubescence, habit of plant, and whether prostrate or erect. This is a report of a monographic study begun at the Arnold Arboretum of Harvard University in 1938, and based, in part, upon an examination of nearly 5,000 sheets of preserved material from the

principal North American herbaria.

Hedyotis (Rubiaceae) and its relatives in america. F. R. Fosberg, Bureau of Plant Industry, Washington, D. C.—A preliminary survey of generic relationships within the tribe Hedyotidae (Rubiaceae) indicates three possible treatments: (1) To recognize all apparently natural groups of species as distinct genera. (2) To recognize the major ones as genera, appending the smaller or less distinct groups wherever they do least violence to the generic lines. (3) To consider the whole aggregation, excepting certain very aberrant groups, as a single large genus, Hedyotis. The various subordinate groups would then be arranged as subgenera, sections, etc., in as nearly a natural, phylogenetic scheme as possible.

For various reasons, principally lack of profound structural differences between the groups, the latter course is here adopted. Accordingly, the American groups, segregated by various authors as Oldenlandia, Kohautia, Clavenna (Lucya), Anotis, Houstonia, Arcytophyllum, and probably Teinsolen, may be reunited with Hedyotis. Dentella is maintained as a distinct genus. Lipostoma and its recently described segregates Standleya and Bradea, until their obvious connection with Coccocypselum can be investigated with more abundant material, are also maintained as separate. On the basis of these ge-

nera, the position of Coccocypselum in a different tribe is questioned.

Contrary to most recent usage, the name Hedyotis L., according to the International Rules, must be maintained for the aggregate genus, as it was the one selected by J. E. Smith (1811), the first worker to combine Hedyotis L., Oldenlandia L., and Houstonia L. into one genus.

THE AREAS OF CONCENTRATION AND ENDEMISM OF THE NEW WORLD LABIATAE WITH REFERENCE TO THE OLD WORLD CENTERS. Carl Epling, University of California, Los Angeles, Calif.—Such areas are illustrated and tentative conclusions are drawn as to their interrelationships, which appear to lie wholly North and South. The relationship with the Old World centers appears to be across the North Atlantic.

GEOGRAPHICAL DISTRIBUTION OF THE OCHNACEAE WITH SPECIAL REFERENCE TO GENERIC SEGREGATION IN SOUTH AMERICA. John D. Dwyer, Fordham University, New York, N. Y .- The family Ochnaceae is limited to tropical Africa and Asia as well as to tropical America. While the African and Asiatic genera are few, the number of species greatly exceeds those of meridional America. Distributional mapping in South America indicates that the comparatively numerous genera of the Ochnaceae are localized. These narrow distributional centers compare favorably with the structural characters that mark the localized genera. The importance of distributional mapping is essential to an understanding of the framework of this small but scattered family.

Paleontology of plants studied without fossils. Walter T. Swingle, Bureau of Plant Industry, Washington, D. C.—Paleontology is commonly defined as the science of life in past geologic periods, based on the study of fossil organisms, but much can be learned of the ancient status and subsequent evolutionary changes of plants not yet found as fossils by what may be called paleophylogeny.

In connection with a study of all known genera and species of the citrus subfamily (Aurantioideae-Rutaceae) I found that several genera related to Citrus reached Australia some 25 million years ago while it still had land connection to the north, and we can follow their evolutionary development since then. Micro-citrus, closely related to Citrus, is widely distributed in northeastern Australia and has one species in near-by New Guinea. The four Australian species of this genus represent three very distinct lines of development. Another related Australian genus, Eremocitrus, has, since the Late Cretaceous or Early Eocene, become, under semi-arid climatic conditions, a pronounced xerophyte, strikingly different from Citrus in appearance but still able to hybridize freely with it. Other genera of Aurantioideae, and of other plant families when studied in paleophylogenetic perspective, even without fossil remains, can be restored substantially as they were tens of millions of years ago; we can even see how they have evolved subsequently at strikingly different speeds.

CONTINENTAL DISPLACEMENT AND ITS RELATION TO THE FLORAS OF NORTH AMERICA. W. H. Camp, New York Botanical Garden, New York, N. Y.— Although Continental Displacement is as yet incompletely understood and subject to a different interpretation both as to mechanics and sequence of events from that of the original Wegener hypothesis, it affords the botanist a certain comfort in his study of the broader aspects of plant distribution not found in the doctrine of "land bridges."

It is held that the South American-African land masses were sufficiently close to be mutually communicative during the Mesozoic and that the majority of the basic families of flowering plants evolved there (particularly in South America) during that time; that the union between these closely associated land masses and Holarctica (the single Asia-North America-European mass), just prior to the Cretaceous, permitted the migration of these plants into what is now the Northern Hemisphere. The northward displacement of the earth's major land masses explains the presence of tropical and sub-tropical plant fossils in the Arctic, as well as the Pleistocene glaciations.

It is further held that Holarctica finally disintegrated during the late Tertiary and that Europe (the eastern segment) broke away from the midportion (North America) and was joined with the western segment (Asia) no earlier than the Pleistocene.

A SYMBOLIC METHOD OF REPRESENTING AND COMPARING CLIMATES FOR PHYTOGEOGRAPHIC STUDIES. Henry T. Darlington, Michigan State College, East Lansing, Mich.—Formulas are developed to indicate the essential characteristics of the climates of various regions. It is shown how these formulas may be used in making comparisons between various phytogeographic areas, such as deciduous forest, narrow sclerophyll, grass lands, etc. The year is divided into a number of intervals and relative temperatures and precipitations are related in such a way as to give a picture of the essential features of the climate that determine the type of vegetation.

WOOD ANATOMY AND TAXONOMY IN THE RHUS COMPLEX. Charles Heimsch, Harvard University, Cambridge, Mass.—In Barkley's recent monograph concerning the North American species of the Rhus complex, these plants are grouped under six genera: Actinocheita, Metopium, Malosma, Cotinus, Rhus and Toxicodendron. The anatomy of the secondary xylem gives some support to this grouping. Actinocheita, Metopium, and Malosma are diffuse-porous; the other genera are ring-porous. Only Metopium and Malosma have resin canals in the rays. Metopium shows the most abundant paratracheal parenchyma of all the genera, and it is often banded. In contrast to these diffuse-porous genera which can be distinguished individually, Cotinus, Rhus, and Toxicodendron show great similarity in xylem organization. It would seem that, on the basis of xylem anatomy, these ring-porous genera are on approximately the same level of anatomical specialization. This may be due either to parallelism or close affinity

FLORAL ANATOMY OF THE KALANCHOIDEAE. Albert H. Tillson, U. S. Plant Introduction Garden, Glenn Dale, Md.—The floral anatomy of 28 species of the three genera comprising the subfamily Kalanchoideae (Crassulaceae), was studied in order to determine whether the reduction of Bryophyllum and Kitchingia to Kalanchoe could be justified on the basis of vascular anatomy. In all three genera, each of the four petal traces is adnate to the corresponding antepetalous stamen trace as it leaves the floral axis. In Bryophyllum the petal and stamen traces separate almost immediately, while in Kalanchoe and Kitchingia the petal-stamen bundles remain fused for half the length of the corolla tube. This difference in vascular plans remains constant in all species studied, even though the level of insertion of the stamens varies greatly in each of the genera. The validity of the genera Bryophyllum and Kitchingia is supported by this anatomical evidence.

AN ALKALINE BOG IN NORTHWESTERN IOWA. W. A. Anderson, State University of Iowa, Iowa City.—A hanging bog near Silver Lake, Dickinson County, Iowa, is fed by springs with a high concentration of minerals, and supports a very interesting flora. Over fifty species of plants are listed, of which twelve are extremely rare locally but have wide ranges, especially in the Great Lakes-St. Lawrence Valley re-

gion. Comparison is made with similar local bogs, of which this is one of the richest remaining in the highly cultivated State of Iowa.

Preliminary survey of the flora of grand manan island, new brunswick, canada. E. W. B. Chase, Wayne University, Detroit, Mich.—During the summer of 1938 a plant survey was made on the Island of Grand Manan, lying off the coast of Maine in the Straits of Fundy. A considerable collection was made and identified with the assistance of the Canadian botanist, John Adams, who visited the island later in the summer and checked the plants then in bloom. The geologic formations are briefly discussed together with an outline of the work of previous workers. A list of plants will be given together with a brief discussion of ecological factors. Special mention will be made of the survival of forms brought in in ships' ballast.

RECENT TRENDS IN THE DISTRIBUTION OF WEEDS IN KANSAS. Frank C. Gates, Kansas State College, Manhattan, Kan.—During the past half century the number of plants recognized as weeds in Kansas has risen from 209 to 372, of which 54 were not present 50 years ago. Distinctly northward or southward migrations are shown by less than ten plants each. Thirty-one plants, all but 2 native, have spread eastward. The largest number, over 125, including several native as well as introduced weeds, old timers and new arrivals, are spreading westward, while 116 have remained within the limits they had in the past century.